EFFECTS OF EXOGENOUS ENZYMES ALONE OR IN COMBINATION WITH OLIGOSACCHARIDES ON UTILIZATION OF TOTAL DIETARY FIBER, FERMENTATION, GROWTH PERFORMANCE, AND HEALTH OF WEANLING AND GROWING PIGS

BY

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DISSERTATION

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ABSTRACT

Five experiments were conducted to investigate the effects of exogenous enzymes and the combination of the enzyme xylanase with oligosaccharides on the utilization of dietary fiber, fermentation, digestibility of energy and nutrients, growth performance, and health of weanling and growing pigs. Experiment 1 was conducted to establish a database for soluble dietary fiber (**SDF**), insoluble dietary fiber (**IDF**), and total dietary fiber (**TDF**) in feed ingredients commonly used in animal nutrition, and to test the hypothesis that the difference between calculated and analyzed TDF values was equal to zero. A total of 846 samples, classified in four types (i.e., cereal grains, cereal grains coproducts, oilseeds and oilseed coproducts, and other feed ingredients), were analyzed for dry matter (**DM**) and for IDF and SDF, and TDF was calculated as the sum of IDF and SDF. For each feed ingredient, means and standard deviation were calculated, and proximate components (i.e., ash, crude protein, crude fat, and starch) were added and subtracted from the concentration of DM using values from the literature to obtain a calculated TDF value. Results demonstrated a high correlation between analyzed and calculated values for TDF ($r = 0.96$; $P < 0.001$) indicating that the analyzed TDF values can characterize the dietary fiber fraction of plant-based feed ingredients. The difference between calculated and analyzed values is statistically not different from zero ($P > 0.05$) for cereal grains, cereal grains coproducts, and other feed ingredients, but for oilseed coproducts the analyzed TDF did not account for all fiber fractions ($P < 0.05$). Experiment 2 tested the hypothesis that a novel endo-β-mannanase can be used in diets for pigs for a period of 42 days post-weaning without negatively impacting growth performance, serum chemistry, hematological characters, or organ weights, even in a very high dose was used. Results indicated that pigs fed diets containing β-mannanase did not negatively impact general health and growth of the pigs even if included at a very high

dose. Experiment 3 tested the hypothesis that supplementation of a novel combination of xylanase and β-glucanase in diets for growing pigs increases the apparent total tract digestibility (**ATTD**) of gross energy and TDF, and therefore, increase metabolizable energy (**ME**) in high fiber diets for pigs was tested. Results indicated that pigs fed the high dose of the combination of xylanase and β-glucanase tended to have greater ($P < 0.10$) ATTD of gross energy and had greater ($P < 0.05$) ME compared with pigs fed the control diet. However, no differences were observed in ATTD of IDF, SDF, and TDF. In experiments 4 and 5, the hypotheses that the enzyme xylanase or the combination of xylanase and oligosaccharides (i.e., stimbiotic) added to high-fiber diets improves growth performance and intestinal health of weanling pigs, and the apparent ileal digestibility (**AID**), apparent cecal digestibility (**ACD**), and ATTD of gross energy, IDF, SDF, and TDF of diets fed to growing pigs were tested. Results indicated that weanling pigs fed diets with xylanase or stimbiotic increased ($P < 0.05$) ATTD of nutrients and energy in the late nursery, leading to greater ($P < 0.05$) growth performance after 42 d post-weaning. The diet containing xylanase fed to growing pigs had greater ($P < 0.05$) AID, ACD, and ATTD of gross energy, IDF, and TDF, but diet containing stimbiotic only had greater ($P < 0.05$) AID of gross energy, IDF, and TDF, with no improvements in ACD and ATTD of nutrients and energy. In conclusion, determining dietary fiber fractions such as SDF, IDF, and TDF provides accurate information about the fiber composition of most feed ingredients, except oilseeds and oilseed coproducts. The novel endo-β-mannanase can be used in diets for pigs with negatively impacting the general health and growth of the pigs, even if overdose. The combination of xylanase and β-glucanase added in diets for growing pigs can improve energy utilization by pigs. Xylanase and stimbiotic can improve growth performance of weanling pigs because they increase fiber fermentation and therefore, increase nutrient and energy digestibility in diets for weanling pigs,

but xylanase alone is more effective than stimbiotic to enhance fermentation and improve digestibility of nutrients and energy in growing pigs. Overall, results presented in this dissertation demonstrated that exogenous enzymes have positive effects on digestibility and fermentability across the gastrointestinal tract, as well as utilization of energy, growth performance and health of pigs fed high fiber diets.

Key words: digestibility, dietary fiber, energy, fermentation, growth performance, health, pigs

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CHAPTER 1: INTRODUCTION

Swine production efficiency has been a major area of research, particularly in evaluating feed ingredients, formulations, and nutritional strategies. Animal diets represent 60 to 70% of the total production cost, but animals are sometimes inefficient in converting dietary nutrients into animal products, leading to increased production costs and environmental impact due to energy and nutrient losses (Pomar and Remus, 2019). Therefore, strategies to improve nutrient efficiency in pig production are important to meet the challenges associated with the expected increase in the human population and demand for food, whereas limited cropland, use of natural resources, and environmental concerns are associated with farm animal production.

Among the different nutrients in feedstuffs for pigs, plant carbohydrates are the primary source of energy in diets for pigs (Velayudhan et al., 2015). Carbohydrates are divided into digestible and non-digestible carbohydrates (Bach Knudsen et al., 2012). Digestible carbohydrates include monosaccharides, disaccharides, and starch that may be digested by enzymes secreted in the gastrointestinal tract of the pig, whereas non-digestible carbohydrates are those not digested by enzymes secreted by pigs and, therefore, fermented by the microbiota in the large intestine (Navarro et al., 2019). Dietary fiber, which includes the non-digestible carbohydrates and lignin that are intrinsic and intact in plants (Institute of Medicine, 2001; U.S. Food and Drug Administration, 2016), includes a big group of molecules with complex chemical structures that can vary significantly in their digestibility and energy contribution to the pig (McGhee and Stein, 2020; Lee et al., 2022).

The feed industry has integrated raw materials and coproducts from cereal and oilseed processing that are not usable for human consumption into animal feed. Utilizing these coproducts as ingredients in pig diets aims to reduce feed costs, enhance food security, and

minimize the environmental impact of the pig industry (Shurson, 2017). However, there is a concern about using coproducts because they have variable nutrient composition, especially high dietary fiber content (Serena et al., 2007; Woyengo et al., 2014). Pigs may be fed less than 15% total dietary fiber in the nursery (Weber et al., 2008; Yu et al., 2016) and up to 30% in the growing-finishing phase (Navarro et al., 2018; Lee et al., 2022) without compromising growth performance. However, there is a need to increase the ability of the pig to utilize the energy associated with dietary fiber when high-fiber co-products are used in the diets.

Because energy is the greatest and most expensive component of animal diets, exogenous enzymes, such as xylanases, β-glucanases, and β-mannanases have been developed to increase the hydrolysis of the polysaccharides of the dietary fiber in feed ingredients, potentially releasing oligosaccharides that may be more fermentable by the microbial population in the large intestine of pigs, resulting in increased solubility of fiber, greater fermentation, and greater energy and nutrient absorption by pigs (Nortey et al. 2007; Torres-Pitarch et al., 2019; Recharla et al., 2019). Oligosaccharides are short polymers of sugar residues that contain at least three sugar residues, utilized mainly by the gut microbiota as an energy source, which results in the production of short-chain fatty acids that provide energy to the host when fed to growing pigs (Pan et al., 2019; Liu et al., 2023). This fermentation process can increase feed efficiency and growth performance in pigs (Tsai et al., 2017; He et al., 2020; Petry et al., 2020), as well as have a positive effect on the health status of the pigs by maintaining intestinal microbiome structural homeostasis and improve body immunity (Pan et al., 2019; Pang et al., 2021; González-Solé et al., 2022). Therefore, supplementation of exogenous enzymes and oligosaccharides alone or combined in diets for pigs fed high-fiber coproducts may increase the digestibility of dietary fiber, promote

fermentation, and result in greater growth performance and overall health in growing pigs, but data to confirm this theory are lacking.

Therefore, the research included in this dissertation investigates the impact of exogenous enzymes and fermentable carbohydrates, focusing on their effects on the utilization of dietary fiber, fermentation, digestibility of energy and nutrients, growth performance, feed efficiency, and health of weanling and growing pigs. The objectives of this dissertation are: 1) establish the total dietary fiber analysis as the standard method to describe the fiber content in feed ingredients commonly used in diets for pigs; 2) test the hypothesis that a novel endo-β-mannanase can be used in diets for pigs increasing growth performance post-weaning and without negatively impacting the general health of the of pigs; 3) test the hypothesis that a novel combination of the enzymes xylanase and β-glucanase increases the apparent total tract digestibility of gross energy and total dietary fiber, and therefore, the concentration of metabolizable energy of diets when supplemented in diets with high fiber ingredients for pigs; and 4) test the hypothesis that the enzyme xylanase or the combination of xylanase and oligosaccharides improves growth performance, intestinal health, and the apparent ileal, cecal, and total tract digestibility of dry matter, gross energy, crude protein, and total dietary fiber, and the concentration of digestible energy of diets when supplemented in diets with high fiber ingredients for pigs.

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CHAPTER 2: UTILIZATION OF EXOGENOUS ENZYMES AND OLIGOSACCHARIDES FOR DIETARY FIBER FERMENTATION AND ENERGY PRODUCTION IN DIETS FOR SWINE: REVIEW OF LITERATURE

INTRODUCTION

Feed carbohydrates may be digested by the action of endogenous enzymes or microbial enzymes into absorbable units that animals can utilize. In monogastric animals, the dietary fiber portion of feed ingredients pass mostly undigested throughout the small intestine and become available as a substrate for fermentation by bacteria in the large intestine. The microbiome expresses many enzymes needed for its survival, but all the enzymes needed for complete hydrolysis of fiber are not secreted by the microbial populations in the hindgut, resulting in partial fermentation (Stein, 2019). Therefore, the utilization of dietary fiber in swine diets varies from 0 to 97% and depends on the physicochemical characteristics of the fiber compounds, the concentration in the diet, and the physiological status of the animal (Kerr and Shurson, 2013). Low fermentation of fiber results in increased manure excretion and reduced digestibility of nutrients and energy due to the influence of fiber on luminal viscosity or through encapsulation of nutrients (Pedersen et al., 2007; Jha et al., 2010).

Despite the negative impact on nutrient and energy digestibility, there is increasing interest in adding dietary fiber to pig diets due to its influence on intestinal passage rate and the modulation of microbial populations that may benefit animal performance, health, and welfare (Bach Knudsen et al., 2013; Bedford, 2018). This change is also driven by concerns over cereal grains and oilseeds competition with fuel industries and the intent to reduce feed costs by

including available alternative coproducts from the distillers and milling industries (Jha and Berrocoso, 2015).

The objective of this review is to describe the dietary fiber portion of feed ingredients used in pig diets, characterize the exogenous enzymes available to hydrolyze the glycosidic bonds in dietary fiber and to describe the products of enzymatic activity in the small intestine and of microbial activity in the large intestine. Likewise, results of applying exogenous enzymes and oligosaccharides to diets for pigs will be reviewed and the impact on dietary fiber fermentation, intestinal health, and energy production will be highlighted.

CARBOHYDRATES

Carbohydrates are the most abundant organic components in plants. In diets for pigs, cereal grains or cereal grain coproducts represent around 50 to 85% of the ingredients, which provide a major fraction of carbohydrates that serve as a source of energy to the animal (Bach Knudsen et al., 2013).

Carbohydrates can be classified by their degree of polymerization into monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Five-carbon monosaccharides are known as pentoses, whereas six-carbon monosaccharides are known as hexoses. Hexoses include glucose, fructose, mannose, and galactose, while pentoses include arabinose, xylose, and apiose (NRC, 2012). The primary monosaccharide in diets for pigs is D-glucose, but feed ingredients may also contain other monosaccharides such as D-fructose, D-galactose, L-arabinose, D-xylose, D-mannose and acidic sugars, such as D-galacturonic acid and D-glucuronic acid (Choct, 2015).

Disaccharides consist of two monosaccharides linked by an α- or β- glycosidic bond. In diets for pigs, the most prevalent disaccharides are lactose, sucrose, and maltose (Navarro et al.,

2019). Oligosaccharides are chains of three or more monosaccharides linked together by β-glycosidic bonds and are separated from other fiber components based on their solubility in 80% ethanol (Englyst and Hudson, 1996; Englyst and Englyst, 2005). Feed ingredients such as legumes or oilseed meals contain galacto-oligosaccharides (raffinose, stachyose, and verbascose), yeast contains mannan-oligosaccharides, and some cereal grains contain fructo-oligosaccharides (Navarro et al., 2019).

Starch is usually the most prevalent polysaccharide in diets for pigs, composed of glucose molecules linked by α- glycosidic bonds, forming granules of amylose and amylopectin polymers (Hamaker et al., 2019). Amylopectin is a highly branched polymer with both α-1,4 and α-1,6 glycosidic bonds, whereas amylose consists of non-branched helical chains of glucose residues connected by α-1,4 glycosidic bonds (Ring et al., 1988). Starch is hydrolyzed to glucose by digestive enzymes, but a fraction of starch that may resist digestion in the small intestine is called resistant starch (Tan et al., 2021). Polysaccharides other than starch that are located in the cell as well as in the cell wall are collectively known as non-starch polysaccharides, of which cellulose, non-cellulosic polysaccharides, and pectic polymers are most abundant (Bach Knudsen et al., 2016).

Due to differences in digestion, carbohydrates are also classified into digestible or undigestible carbohydrates. Digestible carbohydrates are those that are hydrolyzed by enzymes of the gastrointestinal tract to monosaccharides that are absorbed in the small intestine and enter the pathways of carbohydrate metabolism. Digestible carbohydrates include disaccharides and starch (Choct, 2015). Undigestible carbohydrates are not hydrolyzed by endogenous digestive enzymes, but they may be partially or totally fermented by microbial enzymes in the large

intestine, forming short-chain fatty acids (**SCFA**) that may be absorbed and contribute to the energy status of the animal (Stipanuk, 2018).

DIETARY FIBER

The term dietary fiber was defined by the Institute of Medicine, Food and Nutrition Board, National Academy of Sciences (2001) as 'non-digestible carbohydrates and lignin that are intrinsic and intact in plants', and by the U.S. Food and Drug Administration (2016) as also 'isolated or synthetic non-digestible carbohydrates that have beneficial physiological effects'. Non-digestible carbohydrates, by definition, include oligosaccharides, resistant starch, and non-starch polysaccharides (Bindelle et al., 2008). Lignin is a large molecular-weight polymer of phenolic compounds rather than a carbohydrate but is also undigestible and often associated with cell wall carbohydrates, and therefore, also included in the definition of fiber (Choct, 2015).

Although "fiber" summarizes a group of non-digestible molecules, not all fibers are identical, and they are classified according to their physicochemical properties. The physicochemical properties include solubility, viscosity, fermentability, water holding capacity, and water binding capacity (Urriola et al., 2013). Solubility differentiates soluble and insoluble dietary fiber by the capacity to dissolve in water or other liquid solutions, or in the enzyme solution of the intestinal tract. Solubility is influenced by the glycosidic linkage between monosaccharides (Cho et al., 1997). Viscosity refers to the ability of the carbohydrate to thicken or form gels in solution and the resistance to flow, influenced by the solubility of fiber (Dikeman and Fahey, 2006); thus, soluble fiber is more viscous than insoluble fiber. Water holding capacity or water binding capacity refers to the amount of water absorbed within the structure of fiber, but water binding capacity describes the amount of water retained in the fiber after stress, such as

centrifugation, pH changes, or particle size reduction has been applied (Urriola et al., 2013). Fermentability refers to the efficiency of gut microbes to hydrolyze fiber, depending on the size and structure of the molecule and the presence of the bacteria populations that produce the enzymes required to hydrolyze the β-glycosidic bonds within monosaccharides (Williams et al., 2017). Interactions among fiber characteristics can affect its fermentability. For instance, soluble fiber with high water binding capacity is more fermentable, whereas insoluble fiber is less fermentable because of the lack of access for intestinal microbes (Canibe and Bach Knudsen, 2002).

Analysis of dietary fiber

Several developed analytical procedures aimed to measure dietary fiber based on the solubility of molecules in different solutions; however, fiber is often defined by the method used to measure it because methods vary in their chemical use to determine solubility and represent fibers with different chemical properties (Fahey et al., 2019).

Crude fiber was the first method used to describe the organic residue that was insoluble in sulfuric acid and sodium hydroxide treatments. The crude fiber analysis was developed at the Weende Agricultural Experiment Station in Germany as part of the proximate analysis (Bach Knudsen, 2014). However, the alkali solubilizes some lignin and phenolic compounds, and the acid dissolves many hemicelluloses. Therefore, these compounds escape the analysis and underestimate the true fiber content by 30–50% (Choct, 2015).

The detergent methods were initially developed for the analysis of forages to determine the fiber fractions, such as acid detergent lignin, acid detergent fiber (**ADF**), and neutral detergent fiber (**NDF**; Van Soest et al., 1991). The remaining insoluble residue after boiling a substance in neutral detergent solution is referred to as NDF (i.e., hemicellulose, cellulose, and

lignin), whereas the residue after boiling a sample in sulfuric acid detergent solution is referred to as ADF (i.e., cellulose and lignin). Acid detergent lignin is obtained by treating the ADF residue with 72% sulfuric acid for 3 h, resulting in the dissolution of cellulose and a residue that corresponds to the lignin fraction (Van Soest et al., 1991). The detergent methods are more accurate than measuring crude fiber. However, it underestimates a significant portion of fiber in cereal grains, oilseeds, and their co-products because it does not include soluble hemicelluloses in the analyzed NDF portion (Bach Knudsen, 2001).

Three well-established methods are currently accepted for measuring total dietary fiber (**TDF**). One is the enzymatic-gravimetric method that uses enzymatic removal of non-cell wall organic materials and then gravimetrically measures the residue corrected for ash and nitrogen (Prosky et al., 1992). The enzymatic-gravimetric method measures the total dietary fiber as a unit (method 985.29; AOAC Int., 2019) or with a slightly modified methodology, as insoluble and soluble portions measured separately (method 991.43; AOAC Int., 2019). The TDF procedure is more time-consuming than the crude fiber and detergent methods (Mertens, 2003), but more accurate in determining the fiber concentration in food and feed ingredients.

The second and third methods are enzymatic-chemical procedures that determine non-starch polysaccharides after enzymatic removal of starch, precipitation by 80% ethanol, acid hydrolysis, and derivatization to individual neutral sugar residues by converting them into alditol acetates and analyzing them using gas-liquid chromatography or high-performance liquid chromatography, as well as measuring uronic acid residues by colorimetry (i.e., Uppsala method and Englyst method; Bach Knudsen, 2001). Both procedures defined TDF as enzyme-resistant polysaccharides plus lignin, but they differ in the acid hydrolytic conditions during the procedure and the Englyst method includes a solubilization step for resistant starch (Theander et al., 1994;

Englyst et al., 2007). The concentration of each individual monosaccharide (i.e., rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose) and uronic acids are divided into soluble and insoluble portions, which allow inferences about the chemical composition of the non-starch polysaccharides. However, the insoluble and soluble dietary fiber concentrations are lower than those determined with an enzymatic-gravimetric method, and the laboratory analysis using the enzymatic-chemical method is more complex, time-consuming, and expensive (Mertens, 2003; Shurson et al., 2021).

The current methods include all non-starch polysaccharides that precipitate in 78 to 80% ethanol, and carbohydrates such as undigestible soluble oligosaccharides are excluded from the analysis (McCleary 2007). Therefore, further modifications of the procedures or individual methods for their analysis have been developed (method 2002.01; AOAC Int., 2019; method 2009.01; AOAC Int., 2019; and method 2011.25; AOAC Int., 2019). These later methods include not only the oligosaccharides, but also resistant starch in the fiber fraction.

Structures of non-starch polysaccharides from feed ingredients

Because non-starch polysaccharides are primarily present in the plant cell walls, cereal grains, cereal grain coproducts, and oilseed meals provide non-starch polysaccharides to diets for pigs (Navarro et al., 2019). Non-starch polysaccharides have been classified into three groups: cellulose, non-cellulosic polysaccharides, and pectic polymers (Choct, 2015).

Cellulose

Cellulose consists of straight chains of β-(1,4)-linked glucose units that can pack tightly in a 3-dimensional structure by hydrogen and Van der Waals bonds (Held et al., 2015). Cellulose is present in all plant feed ingredients, but can differ depending on the crystalline and amorphous domains, which influences its chemical-physical properties. Crystalline regions are highly

organized microfibrils through extensive inter and intramolecular bonds, which make them largely resistant to fermentation (Quiroz-Castañeda and Folch-Mallol, 2013). In contrast, amorphous cellulose is characterized by twists and torsions that alter the ordered arrangement and increase hydrophilicity, reactivity, and enzymatic digestibility; therefore, amorphous regions of the cellulose may be fermented (Ioelovich, 2021).

Non-cellulosic polysaccharides

Non-cellulosic polysaccharides contain numerous polysaccharides, also called 'hemicellulose', because these polysaccharides can be extracted together (Neukom, 1976). Non-cellulosic polysaccharides in cereal grains and cereal grain coproducts include arabinoxylans, and mixed-linked β-glucans, whereas β-mannans and xyloglucans are present in oilseeds and oilseed meals (Lannuzel et al., 2022).

Arabinoxylans are the main non-starch polysaccharide in cereal grains and cereal grain coproducts (Jaworski et al., 2015). Arabinoxylan contains β-(1,4) xylose units linked to form the backbone and sidechains containing arabinose, D-glucuronic acid, and acetyl groups (De Vries and Visser, 2001). In addition, ferulic or coumaric acids can link to arabinoses, which facilitate chelation with other polysaccharides and lignin. D-glucuronic acid can substitute xylan in the backbone and xylose units may link to arabinose units in the sidechains, which may be further replaced with galactose (Bautil and Courtin, 2019). The arabinose over xylose ratio (**A/X**) reflects the structural features of the arabinoxylans and is used as an indicator of fermentability because arabinoxylans with less branching or lower A/X are more readily fermented than highly branched or substituted arabinoxylans (Tiwari et al., 2019). For example, wheat arabinose residues are single side-chain mono-substitutions or di-sustitutions, with an A/X between 0.57 and 0.70 of mostly insoluble arabinoxylans (Buksa et al., 2016), whereas corn arabinoxylans are

more substituted and form intertwined structures with cellulose or lignin, and the A/X for corn is 0.81 (Petry and Patience, 2020).

Mixed-linked β-glucans are chains of glucose units in a mixture of β-(1,4) and β-(1,3) linkages, with blocks of (1-4) linked units (oligomeric cellulose-like segments) separated by (1-3) linkages (Izydorczyk, 2017). Beta-glucans are present in quantities lower than arabinoxylans and cellulose in grains. Polished rice, sorghum, and corn contain less than 1% mixed-linked β -glucans, whereas the concentration is approximately 2% in rye, and 2 to 5% in barley and oats (Shewry and Serna-Saldivar, 2023). The structure of mixed-linked β-glucans is an asymmetric conformation that prevents tightly packeting of the glucose chains, but allows the molecule to link water molecules, making it soluble and with the ability to form gels that enhance the viscosity of aqueous solutions (Du et al., 2019). Mixed-linked β-glucans are, therefore, highly fermentable in the hindgut of pigs.

Mannans are mannose units linked by β-(1-4) glycosidic bonds, forming a long chain of only mannose units or a chain of mannose units with side chains of α-1,6-linked galactose, glucose, or galactose and glucose residues, resulting in galactomannans, glucomannans or galactoglucomannans, respectively (Chen et al., 2018). Mannans are contained in the cell wall fraction of leguminous plants and oil seeds, including soybean, sesame, palm kernel, copra, and guar, and in the oilseed meals after oil extraction (Lee and Brown, 2022). Soybean meal contains between 0.7% and 2.1% β-mannans, which are mostly associated with the hull fraction (Kiarie et al., 2021). In cereal grains, the concentration of β-mannans is less than 0.5%. Cereal co-products such as corn gluten meal and wheat middlings have slightly greater concentration of β-mannans than cereal grains, but still less than 1% (Bach Knudsen, 1997). Mannans are also part of yeast cell wall structure as a highly branched polysaccharide of a-(1→6) linked mannose units with a-

(1→2)– and a-(1→3)– linked side chains that may connect to amino acids (Orlean, 2012). Distillers dried grains with solubles have up to 2% β-mannans due to contamination with residual yeast cell wall mannans and greater concentration of dietary fiber (Kiarie et al., 2021).

Xyloglucans consist of a backbone of glucose units linked by β-(1-4) bonds as cellulose, but with sidechains rich in xylose. The xylose residues can be further substituted with galactose, fucose, and acetyl groups (Pauly and Keegstra, 2016). Xyloglucans form cross-linked microfibrils linked with cellulose through hydrogen bonds that provide structure to the plant cell walls (Pauly and Keegstra, 2016).

Pectic polymers

Pectic polymers include a diverse group of cell wall polysaccharides including homogalacturonan, rhamnogalacturonans I and II, xylogalacturonans, galactans, arabinans and arabinogalactans (Gawkowska et al., 2018). Homogalacturonans consist mainly of unbranched a-(1-4) galacturonan chains found in the cell walls of sugar beet, potato, apple, cotton, watermelon, carrot, pea, banana, peach, and citrus fruits (Mohnen, 1999). Xylogalacturonans comprise a homogalacturonan backbone with β-(1-3)-linked xylosyl residues, found in fruits such as apple and passioin fruit, and also reported in soybean meal at very low amounts (Beldman et al., 2003; Lannuzel et al., 2022). Rhamnogalacturonans are also a-(1-4) galacturonan chains with alternating α-(1-2)- rhamnose residues in the backbone, but also have a-(1-2) rhamnose residues as side chains linked to the backbone. Side chains with galactose, arabinose, xylose, and less frequently, fucose and glucuronic acid can also be present (Lara-Espinoza et al., 2018). Arabinans are composed of a backbone of a-(1-5)-arabinose residues, which can be branched with additional arabinose units (Levigne et al., 2004). The arabinogalactans have a β-(1-4)-galactose backbone, but have arabinose or galactose units as side chains (Ralet et al., 2009).

Rhamnogalacturonans, arabinans, and arabinogalactans are abundant in the fiber portion of oilseed meals, such as rapeseed and sunflower meals. Soybean meal contains 6.8% of pectin polymers, of which rhamnogalacturonans are the most abundant (Navarro et al., 2019; Lannuzel et al., 2022).

CHARACTERIZATION OF EXOGENOUS ENZYMES NEEDED FOR HYDROLYSIS OF DIETARY FIBER AND PRODUCTS OF ENZYMATIC ACTIVITY

Exogenous enzymes of microbial origin have been developed since the 1980s to supplement diets with fibrous ingredients and improve the utilization of dietary fiber, improve nutrient availability, and support animal growth and health, while manure excretion is reduced (Vehmaanperä, 2022). Microbial enzymes currently used as additives in animal diets are produced by extraction after fermentation of moist solid substrates (i.e., bran, paddy straw, and other agricultural waste; Sujani and Seresinhe, 2015). The microorganisms used include bacteria (*Bacillus lentus*, *B. subtilis*, *B. stearothermophils*, *B. amyloliquifaciens,* and *E. coli*), yeast (*Saccharomyces cerevisiae*), and fungi (*Aspergillus niger*, *A. oryzae*, and *Trichoderma longibrachiatum*). In the current market for feed enzymes for monogastric animals, phytases and carbohydrases represent 90%, whereas proteases and lipases represent only 10% of commercially sold enzymes for feed (Adeola and Cowieson, 2011).

Carbohydrases, also known as NSPases, are enzymes that may aid in the hydrolysis of undigestible carbohydrates by reducing the molecular weight of a targeted non-starch polysaccharide (Adeola and Cowiesion, 2011). Carbohydrases are classified within the Carbohydrate-Active enzymes database into five classes based on their activity (Drula et al.,

2022) and within classes also classified into families based on similarities in the genetic sequence and the reaction mechanism (Henrissat, 1991).

1. Glycoside hydrolases (**GH**) are enzymes that hydrolyze glycosidic bonds with either the addition or removal of water in the reaction (Enzyme Commission number (**EC**) 3.2.1.-). Within this category, more than 100 families have been reported. *Endo*-glycoside hydrolases cleave a substrate within the middle of a chain, whereas *exo*-glycoside hydrolases cleave a substrate at the non-reducing end (Davies and Henrissat, 1995).
2. Glycosyltransferases are enzymes that catalyze the formation of the glycosidic linkage to form a glycoside, classified within 90 families (EC 2.4.-). These enzymes use an activated donor sugar, such as a nucleoside diphosphate sugar that contains a phosphate leaving group (Lairson et al., 2008).
3. Polysaccharide lyases cleave uronic acid-containing polysaccharide chains to generate a new reducing end (EC 4.2.2.-). These enzymes are classified into 40 families and represent a complementary strategy to the GH for hydrolysis polysaccharides. In contrast with GH enzymes, polysaccharides lyases cleavage occurs without the usage of a water molecule (Lombard et al., 2010).
4. Carbohydrate esterases are enzymes that release acyl or alkyl groups attached by ester linkage to carbohydrates, facilitating the action of GH on complex polysaccharides (EC 3.1.1.-). The carbohydrate esterases are classified into 20 families (Cantarel et al., 2009).
5. Auxiliary activities class covers redox enzymes that act in conjunction with the other 4 classes. This class includes families of lignin degradation enzymes and lytic polysaccharide mono-oxygenases, which catalyze the oxidative cleavage of glycosidic bonds in polysaccharides linked to lignin (Levasseur et al., 2013).

Carbohydrases produced in high proportion include xylanases and glucanases, but other commercially available carbohydrases include β-mannanases, α-galactosidases, and pectinases (Vehmaanperä, 2022). All feed-additive carbohydrases are in the *endo*-GH class, which hydrolyze carbohydrate polymers to generate decreased molecular weight oligo- or polysaccharides (Adeola and Cowieson, 2011; Vehmaanperä, 2022).

Xylanases

Xylanases (endo-1,4-β-xylanase, EC 3.2.1.8) hydrolyze β-(1-4) glycosidic bonds within xylose units of arabinoxylans molecules (Collins et al., 2005). The most common xylanases belong to GH10 and 11 families (Morgan et al., 2017). Xylanases in the GH11 family hydrolyze glycosidic bonds in the xylan backbone between three unsubstituted xyloses, acting principally on the xylose unit at the center of the oligosaccharide chain, and produce large chain-length arabinoxylo-oligosaccharides. In contrast, GH10 xylanases can hydrolyze glycosidic bonds from the reducing end of the substituted residue and require only two unsubstituted xylose units between the branches, producing smaller xylo-oligosaccharides (Biely et al., 1997; Morgan et al., 2017; Nordberg Karlsson et al., 2018). In addition, GH10 xylanases can further hydrolyze the products of the GH11 xylanases (Collins et al., 2005; Vehmaanperä, 2022). Xylanases from the GH11 family are more efficient than the GH10 family enzymes to degrade insoluble arabinoxylans, but both can efficiently hydrolyzed soluble arabinoxylans (Courtin and Delcour, 2001).

Glucanases

Endo-β-glucanases release disaccharides or glucan-oligosaccharides as the main final products, and three types are available depending on the glycosidic bond they hydrolyze: β-(1-4) glucanases (EC 3.2.1.4), β-(1-3) glucanases, and β-(1-3,1-4) glucanases (EC 3.2.1.6; Caseiro et

al., 2022). β-(1-4) glucanases are enzymes in the GH9 family that hydrolyze the linkage between two adjacent glucose units commonly found in the cellulose structure (Perrot et al., 2022). However, cellulose degradation requires additional enzyme activities, like cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21; Horn et al., 2012). Likewise, xyloglucan endohydrolases (EC 3.2.1.151) are enzymes that cleave the glucose chain as β-(1-4) glucanases, but from the backbone of xyloglucans (Eklöf et al., 2010).

β-1,3-glucanases are classified together with β-(1-3,1-4) glucanases in the GH17 family. β-(1-3) glucanases (EC 3.2.1.39) hydrolyze glycosidic bonds between glucose units linked from C-1 to C-3 in linear or partially branched glucose chain backbone of cell wall polysaccharides of plants, algae, and fungi (Balasubramanian et al., 2012). β-(1-3,1-4) glucanases (EC 3.2.1.6) hydrolyze both β-(1-3) and β-(1-4) bonds in β-glucans only when a glucose residue towards the non-reducing end of the substrate is itself substituted at C-3 (Vehmaanperä, 2022). The β-(1-3,1-4) glucanases are involved in degrading mixed-linked β-glucans of cereal grains (Perrot et al., 2022).

Mannanases

Endo-1,4-β-d-mannanases (EC 3.2.1.78) are enzymes of the GH5 family that randomly hydrolyze the linkage between mannose units similarly to glucanases and xylanases but produce short manno-oligosaccharides such as mannobiose and mannotriose (Malgas et al., 2015). For the total hydrolysis of manno-oligosaccharides, enzymes such as exo-β-mannosidases (EC 3.2.1.25), β-glucosidases, α-galactosidases, and acetyl mannan esterases are required to remove the side chain substituents (Chauhan et al., 2012).

α-Galactosidases

α-Galactosidases (EC 3.2.1.22) are enzymes from the GH27 or 36 family, that hydrolyze the α-(1-6) bond of the terminal non-reducing galactose residues in galacto-oligosaccharides (i.e., raffinose, stachyose, and verbascose) and galactomannans (Jindou et al. 2002; Chauhan et al., 2012). The GH27 α-galactosidases have greater substrate specificity for galactose substituents on galactomannan polymers and oligosaccharides, whereas GH36 galactosidases have restricted substrate specificity to small galactose-containing oligosaccharides (Wang et al., 2010).

Pectinases

Pectinases are mixed enzymes that hydrolyze pectin polysaccharides. Because pectin is a complex polysaccharide, three types of enzymes are classified as pectinases and must work continuously for pectin degradation (Haile and Ayele, 2022). First, protopectinases degrade insoluble pectin to polymerized soluble pectin with methoxyl ester groups attached. Pectin methylesterases catalyze the de-esterification of the methoxyl groups on the galacturonic acid backbone, resulting in galacturonic acid chains called pectin acid. Polygalacturonases hydrolyze the α-(1-4) glycosidic bond between galacturonic acid in pectin acid units, generating pectin oligosaccharides. Lastly, polygalacturonate lyase cleaves α-1,4-glycosidic linkage in oligosaccharides, resealing galacturonic acid units (Garg et al., 2016).

EXOGENOUS ENZYMES AND OLIGOSACCHARIDES IN DIETS FOR PIGS

Supplementation of exogenous enzymes has been extensively used in animal feed to increase nutrient digestibility and improve feed efficiency through various modes of action (Bedford, 2018).

Release of trapped nutrients

Exogenous enzymes may release encapsulated nutrients from the cell wall matrix structures, increasing the digestibility of starch, proteins, and fat (Bach Knudsen and Vangsøe, 2019). Addition of exogenous enzymes to diet for pigs (xylanase and β-glucanase) alone or in combination, may improve nutrient digestibility of the diet (Olukosi, et al., 2007; Zhang et al., 2014; Ndou et al., 2015; Zhang et al., 2020). However, the efficiency of exogenous enzymes on nutrient digestibility is inconsistent and variable. No beneficial effect of enzyme supplementation on digestibility of nutrients has been reported (Woyengo et al., 2008; Willamil et al., 2012; Upadhaya et al., 2015; Jerez-Bogota et al., 2020). However, exogenous enzymes aid in controlling post-weaning syndrome in nursery pigs by compensating for the immature endogenous enzyme secretory capacity, resulting in increased nutrient digestibility (Masey O'Neill et al., 2014). Xylanase and β-glucanase increased the apparent ileal digestibility of amino acids when included in diets for pigs post-weaning (Yin et al., 2001; Li et al., 2013). However, inconsistent effects on nutrient digestibility in weanling pigs have also been reported (Torres-Pitarch et al., 2017). It is likely that the effect of carbohydrases varies depending on the ingredients in the diet and the characteristics of the fiber compounds (Abelilla and Stein, 2019; Torres-Pitarch et al., 2019), as well as depending on the microbial source and the purity of the enzymes (Ndou et al., 2015).

Reduction of digesta viscosity

Exogenous enzymes may modify the solubility of fiber fractions, reducing the viscosity and water-holding capacity of the digesta in the small intestine (Masey O'Neill et al., 2014; Bedford, 2018; Bach Knudsen and Vangsøe, 2019). Indeed, carbohydrases reduced viscosity, solubilized fiber portions, and released degradation products in the digesta of pigs in some

experiments (Lærke et al., 2015; Tiwari et al., 2019; Jang et al., 2024), but in some instances no such effects were observed (Passos et al., 2015; Duarte et al., 2021). The viscosity of digesta can be affected by the type of ingredient in the diet and the type of fiber in the ingredients (Willamil et al., 2012). Although the enzyme complex may modify the viscosity of digesta, it can create a negative effect by impairing digestion and absorption, counteracting possible effects of carbohydrases and resulting in the absence of an overall response in digestibility (Hung et al., 2022). Likewise, the viscosity of digesta in pigs is considerably less than in poultry due to the greater concentration of water in pig digesta, and therefore, effects of enzymes on viscosity of pig digesta may be negligible (Bedford and Schulze, 1998).

Prebiotic like-properties and fermentation

The third mode of action of exogenous enzymes is the prebiotic properties of the products of the hydrolysis of complex fibrous compounds (Bedford, 2018; Bach Knudsen and Vangsøe, 2019). Although carbohydrases can modify the structure and physicochemical properties of dietary fiber, they typically do not hydrolyze the fibers completely to monosaccharides; instead, enzymatic activity in the gastrointestinal tract often results in oligosaccharides and small polymers (Morgan et al., 2017). These oligosaccharides include arabinoxylo-oligosaccharides, xylo-oligosaccharides, gluco-oligosaccharides, xylo-oligosaccharides, galacto-oligosaccharides, manno-oligosaccharides, and pectin oligosaccharides, which may have prebiotic-like effects because they are selectively fermented by the intestinal anaerobic microbiota, stimulate the growth and activity of commensal bacterial species in the large intestine, and reduce intestinal pH due to synthesis of SCFA, which suppresses pathogenic bacteria growth, thus conferring benefits upon host health (Patel and Goyal, 2011; Kiernan et al., 2013; Zeng et al., 2023).

Anaerobic microorganisms produce enzymes to degrade oligosaccharides into monosaccharides to use as an energy source (Jha and Berrocoso, 2015). Hexoses are metabolized via glycolysis, whereas pentoses are metabolized via the pentose phosphate pathway, but both pathways result in synthesis of pyruvate, which is then oxidized to lactate or SCFA. The most common SCFA are acetate (C2), propionate (C3), and butyrate (C4; Cook and Sellin, 1998), but hydrogen, carbon dioxide, and methane are also generated during fermentation but excreted with feces (Macfarlane and Macfarlane, 2003). Most of the SCFA generated by microorganisms are absorbed through the intestinal cells via passive or active transport, and they will eventually be transported to the liver via the hepatic portal vein. However, some butyrate is metabolized by colonocytes to produce adenosine triphosphate (**ATP**). In the liver, butyrate and acetate may be used for ATP synthesis or as precursors for fatty acid synthesis, whereas propionate is used to synthesize glucose via gluconeogenesis. A small part of the SCFA are not absorbed and instead are excreted in the feces (Jha and Berrocoso, 2015).

Increased microbial fermentation results in increased digestibility of energy, as has been demonstrated *in vitro* (Smiricky-Tjardes et al., 2003) and *in vivo* in growing pigs (Pan et al., 2019). Supplementation of carbohydrases or prebiotic oligosaccharides may increase the digestibility of energy, ADF, and NDF, and result in greater SCFA concentrations in feces (Zhao et al., 2012; Passos et al., 2015; Lan et al., 2017; Tsai et al., 2017; Tiwari et al., 2018; Chen et al., 2021; Boston et al., 2024).

Intestinal health effects

Carbohydrases and oligosaccharides may indirectly or directly benefit intestinal health by generating substrates for beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*, thereby promoting their growth and enhancing the health benefits associated with these bacteria (Smith

et al., 2010; Xing et al., 2020; Petry et al., 2021). More direct effects of carbohydrases and oligosaccharides include reducing the binding sites available to pathogens, thereby preventing pathogen adhesion, and direct immunomodulation through binding to receptors that regulate cytokine production and T-cell response (Moita and Kim, 2022; Kiernan et al., 2023). Likewise, exogenous enzymes may improve the antioxidant status in pigs by reducing the production of free radicals that can result in damage to cell structures. Enzymes may also improve intestinal morphology by increasing villus height, and increase the barrier function by increasing the expression of tight junction proteins (Tiwari et al., 2018; Duarte et al., 2019; Petry et al., 2020; Boontiam et al., 2022).

Effects on growth performance

Pigs fed diets containing exogenous enzymes may have increased overall growth performance because exogenous enzymes provide other benefits for intestinal health (He et al., 2020, Jo et al., 2012). However, improvements in average daily gain, feed intake or feed efficiency of weanling and growing-finishing pigs have not always been observed when carbohydrases were included in the diets (Torres-Pitarch et al., 2017; 2019). There is, therefore, a need to conduct research to identify the barriers to positive responses to the use of carbohydrases in diets for pigs.

STIMBIOTICS

The concept of a stimbiotic was proposed by González-Ortiz et al. (2019) under the premise that oligosaccharides with prebiotic-like effects increase SCFA production when fed to poultry or pigs. Stimbiotics are the products of enzymatic activity, produced industrially by fermentation technologies, and fed to animals at low doses (i.e., as low as 50 g/t; Morgan et al.,

2023), that act as fiber utilization enhancers because they signal to fiber fermenting bacteria to increase their activity and thereby promote an increase in fiber fermentation, but contribute little to the production of SCFA through direct fermentation (Ribeiro et al., 2018; Kiernan et al., 2023). Xylo-oligosaccharides are currently the only recognized stimbiotic, and further exploration is warranted to identify additional stimbiotics.

In contrast to prebiotics, stimbiotics may be added alone or in combination with exogenous enzymes in diets for pigs or poultry, and stimbiotics improve the performance of broilers more than xylanase alone (González-Ortiz et al., 2019). In pigs, stimbiotics have been reported to reduce inflammatory markers, and improve intestinal barrier function, SCFA production, and antioxidant function (Yin et al., 2019; Pan et al., 2019; Hou et al., 2020), but effects on growth performance have been inconsistent (Liu et al., 2018; Yin et al., 2019; Sutton et al., 2021; Chen et al., 2023). There is, however, limited information about the interactions between exogenous enzymes and stimbiotics if added to the same diets for weanling and growing-finishing pigs. Therefore, additional work is needed to determine effects of exogenous enzymes in combination with stimbiotics in diets for pigs. Information about the mechanism of action of each product, the fermentation of dietary fiber, the length of time it will take for enzymes to improve energy and nutrient digestibility, doses, characteristics, concentration of enzyme and stimbiotic, and optimal conditions to be activated are also needed.

CONCLUSIONS

Dietary fiber provide a significant amount of energy to diets for pigs and supplementation of exogenous enzymes and oligosaccharides may enhance the potential benefits of dietary fiber. The physicochemical composition of fiber in feed ingredients is variable, and a thorough chemical analysis of fibrous feed ingredients is necessary. The inconsistent effects of exogenous

enzyme supplementation on nutrient utilization and growth performance in pigs can be attributed to the variability in enzyme types, concentrations, and the specific feed ingredients used because not all enzymes target the same substrates. Additionally, the age, genetics, and health status of pigs influence their response to carbohydrases. Therefore, specific enzymatic mechanisms to facilitate the hydrolysis of glycosidic bonds are needed to enhance fermentation, improve intestinal health, and optimize synthesis of energy. The detailed specificity of enzymes alone or combined with multiple enzymes to hydrolyze targeted glycosidic bonds also deserves further consideration.

Stimbiotics used alone or in combination with exogenous enzymes may modulate hindgut microbiota and improve fermentation of dietary fiber, which may result in a positive effect on nutrient digestibility and growth performance. However, research is needed to understand the possible interactions between stimbiotics, carbohydrases and the microbiome in the gastrointestinal tract of pigs, and future experiments also need to explore possible synergistic effects. This will facilitate the development of more efficient and sustainable feeding strategies within the swine industry.

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CHAPTER 3: SOLUBLE DIETARY FIBER, INSOLUBLE DIETARY FIBER, AND TOTAL DIETARY FIBER IN FEED INGREDIENTS USED IN DIETS FOR PIGS

ABSTRACT

Dietary fiber is defined as the undigestible carbohydrates and lignin fractions of plant-feed ingredients. The most complete and representative analysis of fiber is the total dietary fiber (**TDF**) analysis, which includes the soluble dietary fiber (**SDF**) and the insoluble dietary fiber (**IDF**). There is, however, a lack of data for concentrations of SDF, IDF, and TDF in feed ingredients commonly used in diets for pigs. Therefore, work was conducted to quantify fiber fractions of plant feed ingredients to establish a database for SDF, IDF, and TDF in feed ingredients commonly used in animal nutrition. A total of 846 samples were analyzed for dry matter (**DM**) and for IDF and SDF and TDF was calculated as the sum of IDF and SDF. Analyzed values for SDF, IDF, and TDF were corrected to 88% DM. For each feed ingredient, means and standard deviation were calculated, and proximate components (i.e., ash, crude protein, crude fat, and starch) were added and subtracted from the concentration of DM using values from the literature to obtain a calculated TDF value. The hypothesis that the difference between calculated and analyzed TDF was equal to zero was tested using PROC MIXED of SAS. Results demonstrated a high correlation between analyzed and calculated values for TDF (r = 0.96; $P < 0.001$) indicating that the TDF values obtained using the enzymatic-gravimetric method can characterize the dietary fiber fraction of plant-based feed ingredients. Likewise, results demonstrated that the TDF values obtained by the enzymatic-gravimetric method account for the majority of compounds in the dietary fiber fraction of plant-based feed ingredients, as the difference between calculated and analyzed values is statistically not different from zero ($P >$

0.05) for cereal grains, cereal grains coproducts, and other feed ingredients. However, for oilseed coproducts the analyzed TDF did not account for all fiber fractions ($P < 0.05$), which likely is due to the presence of soluble oligosaccharides and other low-molecular weight sugars in these ingredients. In conclusion, determining dietary fiber fractions as SDF, IDF, and TDF provides information about the fiber composition of feed ingredients, which can be used to improve information about the energy value of feed ingredients.
Keywords: dietary fiber, feed ingredients, pigs.

Abbreviations: DDGS, distillers dried grains with solubles; DM, dry matter; IDF, insoluble dietary fiber; MSC, maximized stillage co-products; SD, standard deviation; SDF, soluble dietary fiber; TDF, total dietary fiber

INTRODUCTION

Complete and representative chemical composition of feed ingredients is crucial for determining the nutritional value that can be used in diet formulation to support optimal digestion, nutrient absorption, and gut health in animals (NRC, 2012; Navarro et al., 2018). Traditionally, swine diets have been formulated based on grains that mainly contribute energy, and oilseed meals that provide amino acids in the diets (Stein et al., 2016). However, these ingredients may be replaced with co-products to provide similar nutrient profiles, which may reduce diet costs (Zijlstra and Beltranena, 2013). Most co-products contain more dietary fiber than cereal grains and oilseed meals, which makes these ingredients less expensive (Anguita et al., 2006) and may be fed to pigs without affecting growth performance (Widmer et al., 2007; Yu et al., 2016; Casas et al., 2018; Acosta et al., 2021) although that is not always the case

(Woyengo et al., 2014). Dietary fiber is defined as undigestible carbohydrates and lignin fractions of plant-feed ingredients (Navarro et al., 2019). Fiber can be quantified and characterized as crude fiber, neutral detergent fiber, acid detergent fiber, total dietary fiber (**TDF**) or non-starch polysaccharides plus lignin (Figure 3.1). Among the methods available, the most representative, robust and reproducible method is determining TDF by adding the values of the soluble dietary fiber (**SDF**) and insoluble dietary fiber (**IDF**) fractions (Mertens, 2003; Fahey et al., 2019). Data for crude fiber and detergent fiber in most feed ingredients have been published (Sauvant et al., 2004; NRC, 2012; Stein et al., 2016), but there is no comparable database for concentrations of SDF, IDF, and TDF in ingredients commonly included in diets for pigs. Therefore, the present work was conducted to establish a database for SDF, IDF, and TDF in feed ingredients commonly used in animal nutrition, and to provide information about the quantities of different fiber fractions that each ingredient provides.

MATERIALS AND METHODS

Description of samples

A total of 846 ingredient samples were collected from commercial feed mills around the world. Suppliers provided approximately 1 kg of each ingredient. From the samples, 554 samples were analyzed at the Monogastric Nutrition Laboratory at the University of Illinois at Urbana Champaign, IL, USA, whereas 292 samples were analyzed at Trouw Nutrition (Boxmeer, The Netherlands). Identical procedures were used to analyze the samples in the two laboratories.

Ingredient samples were classified in 4 groups: cereal grains, cereal grains co-products, oilseeds and oilseed co-products and other feed ingredients. Cereal grains included barley, corn, extruded corn, high-oil corn, oats, rice, rye, sorghum, and wheat. Cereal grain co-products

included bakery meal, barley rootlets, corn co-products (i.e., corn bran, distillers dried grain with solubles (**DDGS**), corn fermented protein, corn germ meal, corn gluten feed, corn gluten meal, and corn starch), oat hulls, rice coproducts (i.e., rice bran, broken rice, rice flour, rice mill feed, and rice protein), sorghum DDGS, and wheat coproducts (i.e., wheat bran, wheat DDGS, wheat flour, wheat gluten feed, and wheat middlings). Oilseeds and oilseed co-products such as 00-rapeseed expellers, 00-rapeseed meal; canola expellers, canola meal, copra expellers, copra meal, cottonseed meal, palm kernel expellers, palm kernel meal, full-fat soybeans, soy protein concentrate, soy protein isolate, soybean expellers, soybean meal, fermented soybean meal, enzyme-treated soybean meal, soybean hulls, sunflower expellers, sunflower meal, and sunflower protein concentrate were also included. Other ingredients included alfalfa meal, chicory pulp, faba beans, field peas, flaxseed meal, lupins, pea flakes, pea protein concentrate, pea starch, pectin, pistachio, pistachio blanks, pistachio shell powder, potato protein concentrate, and sugar beet pulp.

Chemical analysis

Samples were finely ground through a 0.5 mm screen and analyzed for dry matter (**DM**) determined by oven drying at 135 ºC for 2 hours (method 930.15, AOAC Int., 2019). Samples were also analyzed for IDF and SDF using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) as established by method 991.43 (AOAC Int., 2019). Briefly, 0.5 g of each sample was weighed in duplicate and placed into filter bags designed to hold the sample during enzymatic digestion with α-amylase, protease, and amyloglucosidase. After digestion, the mixture was filtered. The insoluble residue remained in the filter bags whereas the soluble residue was in the filtrate. The soluble residue in the filtrate was precipitated using 78% ethanol and remained in the filter bags. Both the insoluble and soluble fiber residues were dried

and weighed. One replicate of the insoluble and soluble residue were analyzed for nitrogen by the Kjeldahl method (Method 2001.11; AOAC Int., 2019) using a Kjeltec^{TM} 8400 apparatus (FOSS, Eden Prairie, MN, USA). Crude protein was calculated as 6.25 × nitrogen. The second replicate of the residue was analyzed for ash (method 942.05: AOAC Int., 2019).

Calculations and statistical analyses

The IDF and SDF in each sample was calculated using the following equations (AOAC Int., 2019):

$$\text{IDF (\%)} = \frac{\text{residue}_{\text{insoluble}} - (\text{protein}_{\text{insoluble}} + \text{ash}_{insoluble})}{\text{initial sample weight}} \times 100$$

where $\text{residue}_{\text{insoluble}}$ is the average weight of the dried insoluble residue after the first filtration of the replicates for each sample (g), $\text{protein}_{\text{insoluble}}$ is the crude protein content in the insoluble residue (g), and $\text{ash}_{insoluble}$is the ash content in the insoluble residue (g), and $\text{initial sample weight}$ is the average of the initial weight of the replicates for each sample.

$$\text{SDF (\%)} = \frac{\text{residue}_{\text{soluble}} - (\text{protein}_{\text{soluble}} + \text{ash}_{\text{soluble}})}{\text{initial sample weight}} \times 100$$

where $\text{residue}_{\text{soluble}}$ is the average weight of the dried soluble residue after the second filtration of the replicates for each sample (g), $\text{protein}_{\text{soluble}}$ is the crude protein content in the soluble residue (g), and $\text{ash}_{\text{soluble}}$ is the ash content in the soluble residue (g), and initial sample weight is the average of the initial weight of the replicates for each sample. Total dietary fiber was calculated as the sum of IDF and SDF. To allow for statistical comparison among ingredients, the analyzed IDF, SDF, and TDF were adjusted to an 88% DM basis. If two or more samples from an ingredient were analyzed, the average of samples within each feed ingredient and the standard deviation were calculated using Microsoft Excel functions AVERAGE and STDEV.S.

For each feed ingredient, the calculated TDF value was estimated according to the following equation:

$$\mathrm{TDF_{Calculated}} = \mathrm{DM} - (\mathrm{ash} + \mathrm{protein} + \mathrm{fat} + \mathrm{starch})$$

where values of ash, crude protein, crude fat, and starch (%) reported by the NRC (2012) or by the feed ingredient database (Stein, 2025) were subtracted from DM (%) to estimate TDF (%). Calculated TDF values were also adjusted to an 88% DM basis.

The difference between calculated and analyzed TDF values was calculated as:

$$\Delta\mathrm{TDF} = \mathrm{TDF_{Calculated}} - \mathrm{TDF_{Analyzed}}$$

The paired Student's t-test was used to test the hypothesis that the difference between calculated and analyzed TDF (i.e., delta TDF) was equal to zero, using PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA). The type of ingredient (i.e., cereal grains, cereal grain co-products, oilseeds and oilseed co-products, and other feed ingredients) was the fixed effect. Normality of residuals was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC, USA) and homogeneity of variance was also confirmed. Treatment means were calculated using the LSMEANS statement, and means were separated using the PDIFF statement with Tukey's adjustment. The correlation coefficient between calculated and analyzed values was tested using PROC CORR of SAS and the PEARSON statement. Statistical significance was considered at $P < 0.05$.

RESULTS

Fiber in cereal grains

In cereal grains, values for TDF were in the range of 3% to 35% (Table 3.1). Barley contained 19.67 ± 2.20% TDF with 16.06 ± 2.01% IDF and 3.61 ± 0.77% SDF, whereas dehulled

barley contained 12.64% TDF, 9.38% IDF, and 3.26% SDF. Corn contained 11.81 ± 3.75% TDF with 10.54 ± 2.17% IDF and 1.27 ± 2.42% SDF; however, if extruded, the TDF in corn was 10.00 ± 1.20%, IDF was 8.63 ± 0.49%, and the SDF was 1.37 ± 1.19%. High-oil corn contained 15.04% TDF, which was all IDF. Oats contained 35.06 ± 3.85% TDF, which corresponded to 31.96 ± 4.32% IDF and 3.10 ± 0.54% SDF, whereas extruded oats contained 28.67% TDF, which corresponded to 24.77% IDF and 3.90% SDF. If dehulled, oats contained 12.39 ± 2.34% TDF, reducing IDF to 8.11 ± 2.32% and increasing SDF to 4.28 ± 0.30%. In contrast, brown rice contained only 3.52 ± 0.06% TDF with 3.29 ± 0.04% IDF and 0.23 ± 0.10% SDF. Rye contained 18.89 ± 4.02% TDF, 14.16 ± 1.00% IDF, and 4.73 ± 3.15% SDF, whereas hybrid rye contained 16.57 ± 1.16% TDF, 13.79 ± 0.81% IDF, and 2.78 ± 0.81% SDF. Among sorghum samples, IDF was in the range of 6.42% to 10.00%, and SDF was in the range of 0.23% to 0.59%, giving TDF in the range of 7.01% to 10.30%. Wheat contained 12.08 ± 1.76% TDF with 10.9 ± 1.52% IDF and 1.18 ± 0.59% SDF.

Fiber in cereal grain co-products

Bakery meal contained 16.65 ± 3.73% TDF, 15.10 ± 3.44% IDF, and 1.55 ± 1.55% SDF (Table 3.2). Barley rootlets contained 30.77 ± 1.86% TDF, which corresponded to 29.15 ± 2.20% IDF and 1.62 ± 0.62% SDF. Corn co-products from the wet milling industry, such as corn bran and corn germ meal, contained 40.82 ± 9.41% and 36.58 ± 4.65% TDF, with 38.46 ± 8.96% and 33.50 ± 5.41% IDF, and 2.36 ± 2.40% and 3.08 ± 1.79% SDF, respectively. Corn gluten feed contained 37.14 ± 6.20% TDF with 34.41 ± 7.68% IDF and 2.73 ± 1.63% SDF. Corn gluten meal contained only 7.47 ± 3.76% TDF, 6.79 ± 3.83% IDF and 0.68 ± 0.44% SDF, and corn starch did not contain TDF. Corn DDGS, obtained after ethanol production in the dry grind process, contained 36.07 ± 3.16% TDF with 33.68 ± 3.13% IDF and 2.39 ± 1.45% SDF, but if the DDGS

was further processed by the maximized stillage co-products (**MSC**) system, DDGS contained 38.91 ± 8.30% TDF with 36.77 ± 8.04% IDF and 2.14 ± 0.26% SDF. High protein DDGS contained 37.19 ± 2.12% TDF with 34.18 ± 1.87% IDF and 3.01 ± 0.97% SDF; however, corn fermented protein contained 30.14 ± 1.70% TDF with 28.50 ± 1.21% IDF and 1.64 ± 0.91% SDF. Oat hulls contained 67.61 ± 5.01% TDF, which corresponded to 66.36 ± 4.61% IDF and 1.25 ± 0.39% SDF. Co-products from rice processing, such as full-fat rice bran and defatted rice bran contained 30.90% and 23.32% TDF, with 27.09% and 21.87% IDF and 3.80% and 1.45% SDF, respectively. Broken rice contained 2.28 ± 0.63% TDF with 1.97 ± 0.46% IDF and 0.17 ± 0.20% SDF. In contrast, rice mill feed contained 53.50% TDF, 51.72% IDF, and 1.78% SDF. Rice protein and rice flour contained 6.71% and 1.25 ± 0.50% TDF, with 6.53% and 1.25% IDF, respectively, but these ingredients contained no SDF. Sorghum DDGS contained 36.18 ± 5.80% TDF with 32.08 ± 4.76% IDF and 4.10 ± 0.97% SDF, whereas wheat DDGS contained 28.27 ± 1.88% TDF, 24.91 ± 0.63% IDF, and 3.36 ± 1.43% SDF, and wheat gluten feed contained 30.70 ± 3.04% TDF with 28.42 ± 2.30% IDF and 2.28 ± 0.75% SDF. Wheat flour contained 4.61 ± 2.02% TDF with 2.65 ± 0.63% IDF and 1.96 ± 1.40% SDF. In contrast, wheat bran contained 38.99 ± 3.61% TDF with 36.54 ± 3.57% IDF and 2.45 ± 0.70% SDF, whereas wheat middlings contained 38.02 ± 4.82% TDF with 35.42 ± 4.84% IDF and 2.60 ± 0.72% SDF.

Fiber in oilseeds and oilseed co-products

The TDF in oilseeds and oilseed co-products ranged from 3% to 68% (Table 3.3). 00-rapeseed meal contained 32.14 ± 2.33% TDF, 29.79 ± 2.31% IDF, and 2.37 ± 0.73% SDF, whereas 00-rapeseed expellers contained 35.76 ± 1.43% TDF with 32.07 ± 1.89% IDF and 3.72 ± 0.78% SDF. However, if 00-rapeseed expellers were fermented, they contained 28.47% TDF, 26.10% IDF, and 2.37% SDF. Canola expellers contained 31.23 ± 0.85% TDF, with 26.72 ±

0.49% IDF and 4.51 ± 1.34% SDF, whereas canola meal contained 30.79 ± 3.33% TDF with 27.72 ± 2.96% IDF and 3.08 ± 1.78% SDF. Copra expellers contained 45.14 ± 3.48% TDF, 40.85 ± 1.95% IDF, and 4.29 ± 2.02% SDF, whereas copra meal contained 42.71 ± 3.80% TDF, 38.69 ± 3.61% IDF, and 4.02 ± 0.87% SDF. Cottonseed meal contained 26.80% IDF and 3.00% SDF, giving a TDF of 29.80%. Palm kernel expellers contained 63.50% TDF, 60.90% IDF, and 2.60% SDF, whereas palm kernel meal contained 61.26 ± 4.54% TDF, 58.57 ± 5.06% IDF, and 2.68 ± 0.66% SDF. Full-fat soybeans contained 22.88 ± 4.03% TDF with 20.42 ± 3.53% IDF and 2.46 ± 1.42% SDF, but if fermented, they contained 20.73 ± 0.08% TDF with 16.27 ± 0.07% IDF and 4.46 ± 0.02% SDF. If oil is extracted from soybeans using mechanical pressing, soybean expellers are produced, and they contain 20.55 ± 1.73% TDF, 17.23 ± 1.18% IDF, and 3.32 ± 1.54% SDF, but if oil is extracted using solvents, soybean meal is produced, which contains 18.23 ± 3.28% TDF, 16.01 ± 3.02% IDF, and 2.22 ± 2.25% SDF. Enzyme-treated soybean meal contained 17.24 ± 1.51% TDF, 15.16 ± 0.95% IDF, and 2.08 ± 1.34% SDF, whereas fermented soybean meal contained 17.41 ± 3.36% TDF, 14.78 ± 3.09% IDF, and 2.63 ± 1.38% SDF. If soybean meal is processed by removing soluble carbohydrates, soy protein concentrate is produced, which contains 19.61 ± 3.27% TDF, 17.66 ± 2.95% IDF, and 1.95 ± 1.46% SDF, and if further processed to increase protein concentration, soy protein isolate is produced. This ingredient contains 2.80 ± 1.43% TDF, 2.33 ± 1.70% IDF, and 0.47 ± 0.27% SDF. Soybean hulls contained 67.82 ± 1.98% TDF, which corresponded to 62.24 ± 2.27% IDF and 5.58 ± 1.75% SDF, but if extruded, soybean hulls contained 67.53 ± 0.26% TDF with 60.22 ± 0.23% IDF and 7.31 ± 0.03% SDF. Sunflower expellers contained 40.99% TDF, with 36.87% IDF and 4.12% SDF, whereas sunflower meal contained 43.41 ± 6.39% TDF with 39.56 ± 6.65% IDF and 3.85 ±

2.27% SDF, and sunflower protein concentrate contained 5.27%, with 2.68% IDF and 2.59% SDF.

Fiber in other plant-based feed ingredients

The TDF in other plant-based feed ingredients was in the range of 3% to 82% (Table 3.4). Alfalfa meal contained 49.48% IDF and 2.90% SDF, giving a TDF of 52.38%. Flaxseed meal contained 53.49% TDF with 48.26% IDF and 5.23% SDF. Pulses, such as faba beans, field peas, and lupins contained 16.33%, 15.68 ± 0.83%, and 43.48% IDF and 1.48%, 1.74 ± 0.40%, and 2.76% SDF, giving a TDF of 17.81%, 17.42 ± 1.05% and 46.24%, respectively. If peas are processed, pea flakes may be produced and pea flakes contained 17.31 ± 6.57% TDF, 15.14 ± 4.09% IDF, and 2.17 ± 2.48% SDF, whereas pea protein concentrate contained 8.68 ± 2.44% TDF, 7.57 ± 1.62% IDF, and 1.11 ± 0.82% SDF, and pea starch contained 2.38 ± 1.65% TDF, 2.20 ± 1.52% IDF, and 0.18 ± 0.25% SDF. Chicory pulp contained 47.00% IDF and 15.21% SDF, giving a TDF of 62.21%, whereas sugar beet pulp contained 64.01 ± 7.05% TDF, 49.11 ± 6.75% IDF, and 14.90 ± 5.23% SDF. Pectin contained 52.94 ± 5.61% SDF, and only 0.53 ± 0.62% IDF, giving a TDF of 53.47 ± 4.99%. Pistachio contained 14.44 ± 1.87% TDF with 13.48 ± 1.54 IDF and 0.96 ± 0.33% SDF, whereas pistachio blanks contained 75.99% TDF with 69.90% IDF and 6.09% SDF, and pistachio shell powder contained 82.25 ± 4.00% TDF with 77.72 ± 8.86% IDF and 4.53 ± 4.86% SDF. Potato protein concentrate contained 5.62 ± 2.31% IDF and 0.10 ± 0.15% SDF, giving a TDF of 5.72 ± 2.36%.

The difference between the calculated and analyzed TDF in oilseed co-products was greater ($P < 0.05$) than zero, but this was not the case for cereal grains, cereal grains co-products, and other feed ingredients where no differences between analyzed and calculated values were observed (Table 3.5). The average calculated TDF for all ingredients was 30.48%, and the

average analyzed TDF was 28.29%, and there was a positive correlation between the calculated and the analyzed values for TDF (correlation coefficient = 0.96; $P < 0.001$).

DISCUSSION

Complete chemical composition analysis of feed ingredients requires accurate measurements of all nutrients and energy-contributing components (Shurson et al., 2021). However, there are no standardized methodologies used to analyze feed ingredients, and the values presented in feed composition tables often do not add up to 100% (Sauvant et al., 2004; NRC, 2012). It is believed that this discrepancy is mainly caused by inaccuracies in analysis of fiber, because some of the available fiber analysis procedures underestimate the fiber concentration in feed ingredients. Indeed, to overcome these difficulties, the fiber in feed ingredients is sometimes calculated rather than analyzed (Blok et al., 2015; Aldenhoven et al., 2020). Among the several methods available to analyze fiber (i.e., crude fiber, detergent fiber, TDF, non-starch polysaccharides plus lignin), the TDF procedure by the enzymatic-gravimetric method is more time-consuming than the crude fiber and detergent methods, but this method is more accurate in determining the fiber concentration in feed ingredients because it includes high molecular weight soluble fiber (Mertens, 2003; Fahey et al., 2019). Compared with calculating the TDF after determining non-starch polysaccharides and lignin via the Uppsala or the Englyst methods, the TDF procedure by the enzymatic-gravimetric method is robust and rapidly reproducible (Mertens, 2003; Shurson et al., 2021; Lancheros et al., 2022) and is particularly valued for its ability to provide consistent results across different laboratories (McCleary et al., 2012; Nguyen et al., 2019).

Results of the present work demonstrated that the TDF values obtained by the enzymatic-gravimetric method account for the majority of compounds in the dietary fiber fraction of plant-based feed ingredients, as the difference between calculated and analyzed values was not different from zero for cereal grains, cereal grains co-products, and other feed ingredients. The accuracy of the TDF method is also demonstrated by the fact that TDF values obtained by this procedure result in low calculated rest fractions in many ingredients, indicating low differences between analyzed proximate components and the concentration of dry matter (Fanelli et al., 2023a; 2023b; 2023c; 2024; Ruiz-Arias et al., 2025). Values for TDF obtained by the enzymatic-gravimetric method include all soluble non-starch polysaccharides that precipitate in 78% ethanol, but the resistant starch and undigestible soluble oligosaccharides that remain soluble in ethanol are excluded from the analysis (i.e., oligosaccharides with a low degree of polymerization (≤ 3 sugar monomers; McCleary, 2023), indicating that these compounds can be equal to those calculated as the rest fraction. However, their exclusion is not critical in cereal grains and cereal grain co-products, because the low-molecular weight fibers are less than 5% in these ingredients. However, the greater difference between calculated and analyzed TDF in oilseed co-products may be due to greater concentration of sucrose and soluble galacto-oligosaccharides, such as raffinose, stachyose, verbascose, which can have up to 15%, and are not analyzed by the enzymatic-gravimetric method (Middelbos and Fahey, 2008; Navarro et al., 2018; Lannuzel et al., 2022; McCleary, 2023). Therefore, to fully account for all nutrients in oilseeds and oilseed co-products, specific analyses for the low molecular weight carbohydrates are needed (Navarro et al., 2018)

The feed industry has integrated plant-based ingredients into animal feed, including raw materials such as cereal grains, oilseeds, legumes, roots, and tubers, as well as co-products from

the human food processing (milling, oil, and sugar extraction), distillery, or industrial processes that result in products that are not usable for human consumption (Ominski et al., 2021). However, these ingredients have variable nutrient composition, and co-products from the food industry often have greater concentrations of dietary fiber (Serena et al., 2007; Woyengo et al., 2014). In monogastric animals, the dietary fiber portion of feed ingredients pass mostly undigested through the small intestine and become available as a substrate for fermentation by bacteria in the large intestine; however, utilization of dietary fiber by monogastric animals varies from 0 to 97% and depends on the activity of the microbiome to hydrolyze and ferment fiber, which also depends on physicochemical characteristics of the fiber compounds, the concentration in the diet, and the physiological status of the animal (Kerr and Shurson, 2013; Stein, 2019). Low fermentation of fiber in monogastric animals results in increased manure excretion and reduced digestibility of nutrients and energy due to the influence of fiber on luminal viscosity or through encapsulation of nutrients (Bachmann et al., 2021; Hung et al., 2022; Lee et al., 2022); but despite these negative impacts, there is an increasing interest in adding dietary fiber to animal diets due to its influence on intestinal motility and gut development, modulation of feed intake and establishment of microbial populations that may benefit animal performance, health, and welfare (Bach Knudsen et al., 2013; Jha et al., 2019; Hu et al., 2023). This change is also a consequence of the increased usage of cereal grains and oilseeds in the biofuels industry and the intent to reduce feed costs by including available alternative co-products in diets for poultry and livestock. Likewise, the increased interest in minimizing the impact of the livestock industry on the external environment by reducing greenhouse gas emissions, land use, and water consumption, and improved sustainability has resulted in greater focus on using co-products in diets (Jha and Berrocoso, 2015; Shurson, 2017; Ominski et al., 2021).

Among cereal grains, oats contained the most TDF and IDF, followed by barley and rye, which is in agreement with previous data (Bach Knudsen, 1997; Rodehutscord et al., 2016; Menkovska et al., 2017; Shewry and Serna Saldívar, 2023). Oats, paddy rice, and barley are harvested with the hull attached, which accounts for the majority of fiber; however, some fiber can also be present in the pericarp and cell walls of the aleurone layer (Serna Saldívar and Sánchez Hernández, 2020), which is in agreement with lower TDF values in dehulled barley, dehulled oats, and brown rice. Rye has greater concentration of TDF compared with corn, sorghum, and wheat, which is in agreement with previous data (Bach Knudsen, 1997; Rodehutscord et al., 2016; Shewry and Serna Saldívar, 2023). Concentration of TDF in sorghum, corn, and wheat are also in agreement with previous data (Bach Knudsen, 1997; Picolli da Silva and Santorio-Ciocca, 2005; Rodehutscord et al., 2016; Menkovska et al., 2017), with most of the fiber consisting of IDF in the pericarp and aleurone layers. The IDF in cereal grains consists mainly of arabinoxylans, as the major polymer in the cell wall, followed by cellulose and lignin (Bach Knudsen, 2014; Jaworski et al., 2015; Navarro et al., 2018). Oats and barley contain more SDF than corn, wheat, and sorghum because of the mixed linked β-glucans that are located in the endosperm and aleurone cell wall of these grains (Bach Knudsen, 2014). Wheat and brown rice also contain β-glucans, but in much lower concentrations than barley, oats, or rye (Lee et al., 2007; Biel et al., 2020). In contrast, rye SDF is composed mainly of soluble arabinoxylans, a polysaccharide made up of a chain of xylose units with sidechains of arabinose, associated with the starchy endosperm (Bach Knudsen and Lærke, 2010). Hybrid rye contains more SDF than conventional rye, which is likely due to the improved grain structure resulting from the hybridization process (Miedaner and Laidig, 2019).

Although among cereal grains co-products there is a wide range in TDF depending on the parts of the grain retained after processing, most of these co-products have greater TDF than cereal grains, mostly IDF and low SDF, and the IDF and SDF reflect the composition in the parent grains (Jaworski et al., 2015). Oat hulls have the most TDF and IDF, which is a result of the fibrous outer layer from the oat grain after dehulling, which is in agreement with previous data (Bach Knudsen, 1997; Flis et al., 2017). Among corn co-products, corn bran, corn germ meal, corn gluten feed, and corn DDGS have high TDF concentration, with IDF as the dominant fraction (Bach Knudsen, 1997; Stein et al., 2016). In contrast, corn gluten meal has low TDF, because it is the product resulting from separating the bran-free corn into the protein fraction and starch by centrifugation (Rausch and Belyea, 2006; Jaworski et al., 2015). Similarly, high-protein DDGS and corn fermented protein are obtained by separating fiber and oil from DDGS or corn before fermentation, resulting in products with higher protein and lower fiber content compared with conventional corn DDGS (Espinosa and Stein, 2018; Acosta et al., 2021). Corn starch is primarily a pure product, obtained after the removal of all other components in corn, which is in agreement with no TDF value reported (Bach Knudsen, 1997; Stein et al., 2016). Barley rootlets are a co-product from the malting industry and have greater TDF than both barley grain and malted barley, because fiber gets concentrated after the removal of starch during fermentation (Neylon et al., 2020). Sorghum DDGS has a concentration of TDF that is not different from corn DDGS, which is in agreement with previous data (Sotak et al., 2014). Rice bran has high TDF because it is mainly composed of the pericarp, aleurone, and germ of the rice kernel, whereas rice mill feed is a mixture of rice hulls and rice bran. However, rice hulls contain mainly lignin and therefore, adding rice hulls results in greater TDF in rice mill feed (Serna Saldívar and Sánchez Hernández, 2020). In contrast, broken rice, rice flour, and rice protein contain very little

TDF, mostly due to the removal of the outer layers (i.e., hulls and the bran) of the rice kernel during processing to separate the starchy endosperm (Casas et al., 2019). Bakery meal consists of food left-overs such as unsalable bread, cookies, dough, flour, cakes, and other products from the food industry; therefore, bakery meal is low in TDF, because wheat flour is the main ingredient in most bakery products (Liu et al., 2018). Among wheat co-products, wheat bran is made of the coarse outer layers of the wheat kernel, whereas wheat middlings contain finer product in a mix of bran, germ, and some endosperm. Therefore, wheat bran and wheat middlings contain more TDF than wheat DDGS and wheat gluten feed, which has also been reported previously (Rosenfelder et al., 2013; Stas et al., 2024).

The fiber in oilseeds is different from fiber in cereal grains and mainly consist of cellulose and pectic polysaccharides (Choct, 2015; Navarro et al., 2019). The variation in the TDF among oilseed co-products is due to differences in the composition of the oilseed, the amount of residual oil in the co-products depending on if solvent extraction or the mechanical press method was used to remove oil, and the amount of hulls added back to the co-product after oil extraction (Lannuzel et al., 2022). 00-Rapeseed, which has been selected to be low in both glucosinolates and erucic acid, is called canola in Canada and the United States and 00-rapeseed in Europe, and meal and expellers from both canola and 00-rapeseed can be used as feed ingredients for animals (Maison et al., 2015). Therefore, the lack of differences in the concentration of TDF between 00-rapeseed meal and canola meal was expected and is in agreement with previous data (Bach Knudsen, 1997; Omotosho et al., 2024). The TDF in copra expellers and copra meal, which is derived from the dried kernel of coconut, as well as in palm kernel meal and palm kernel expellers, are in agreement with previous data, and the fiber in these ingredients is high in β-mannans, xylans, pectins, and cellulose (Düsterhöft et al., 1991; Bach

Knudsen, 1997; Fanelli et al., 2023c). The fiber in cottonseed meal is between 14.5 and 30% (Ma et al., 2018), and the analyzed TDF in this experiment aligns with this range. However, the presence of anti-nutritional factors, particularly gossypol, limits the use of cottonseed meal in diets for monogastric animals. Soybean hulls consist of the soybean seed coat, and the fiber in soybean hulls include cellulose, soluble arabinogalactans and pectic polysaccharides, and TDF, IDF, and SDF obtained in this work agree with previous values (Middelbos and Fahey, 2008). The analyzed TDF in full-fat soybeans is also in agreement with previous data (Middelbos and Fahey, 2008; Ruiz-Arias et al., 2025). Fermentation or enzyme-treatment can reduce the fiber in oilseed co-products, as well as reduce other antinutritional factors, as observed for 00-rapeseed expellers, soybean meal, and full-fat soybeans (Zhu et al., 2023). Processing to purify protein from oilseeds results in the removal of the fiber content; therefore, high-protein ingredients such as soy protein concentrate, soy protein isolate, or sunflower protein concentrate have low TDF concentration, which is in agreement with previous data (Shewry and Serna Saldívar, 2023). Sunflower meal has more TDF than soybean meal and canola meal, due to the high concentration of hulls (Lannuzel et al., 2022).

Other plant-based feed ingredients had a broad range of TDF concentrations, mainly because the TDF reflects both plant origin and different processing characteristics. Pistachio shell powder and pistachio blanks had the greatest TDF among all ingredients analyzed and are composed primarily of cellulose and xylans (Kim et al., 2024). Although high in TDF, pistachio shell powder can be used in diets for sows (Kim et al., 2024). Sugar beet pulp and chicory pulp are high in TDF and have high concentration of SDF due to the high concentration of pectin in the fiber fraction (Bach Knudsen, 1997; de Godoy et al., 2015; Wang et al., 2016). The majority of TDF in field peas, faba beans, and lupins, are present in the seed coat and cell walls of the

cotyledons, and include xyloglucans and cellulose, which are included in IDF (Shewry and Serna Saldívar, 2023). The high TDF in alfalfa meal is due to its leaf and stem structure, whereas the high TDF in flaxseed meal is due to the seed outer layer that consist of cellulose and lignin (Bach Knudsen, 1997; Kajla et al., 2015). Processing into protein concentrates or pure starch reduces fiber, which is the reason for the low TDF in potato protein, pea protein, and pea starch due to the removal of hulls and other fibrous components during fractionation (Messina et al., 2025).

Prediction equations are sometimes used to predict the energy concentration of animal diets and feed ingredients using crude fiber or detergent fiber values (Noblet and van Milgen, 2004; Sung and Kim, 2021). Although neutral detergent fiber can be used for predicting digestible energy in diets for pigs (Choi et al., 2020), this may result in erroneous prediction equations because the soluble fiber, which in not included in the neutral detergent fiber, may contribute energy to the ingredients and diets. To predict the energy value of a feed ingredient, it is important that all energy-contributing components are accounted for (Navarro et al., 2018). Specifically, it may be important to incorporate SDF into prediction equations for energy because SDF is much more fermentable than IDF (Urriola et al., 2010; Jaworski and Stein, 2017). Therefore, additional research is warranted to develop equations incorporating IDF, SDF, and TDF values and to validate them through comparison with *in vivo* experiments.

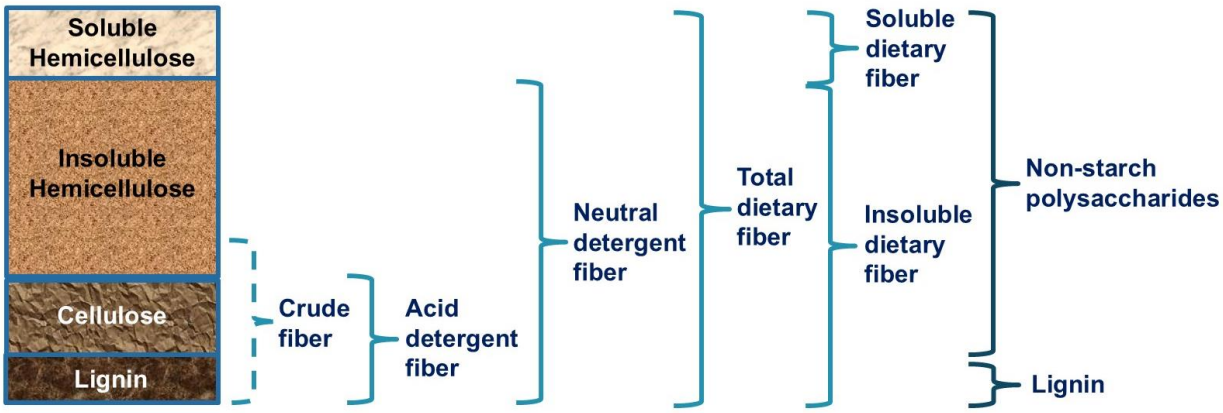
CONCLUSIONS

Describing insoluble and soluble fiber fractions in feed ingredients using the enzymatic-gravimetric method provides information about fiber characteristics that allow for the 100% characterization of the chemical composition of feed ingredients. This work also provides a database that covers the majority of the feed ingredients commonly used in feeding of pigs and

poultry. By distinguishing between soluble and insoluble fiber fractions, improved information about the energy value of feed ingredients can be obtained. The current database is particularly relevant as the use of fibrous ingredients has increased in diets for pigs and other animals. Results also demonstrate that the TDF procedure as applied in this work offers a practical method for fully characterizing fiber in feed ingredients. More research is recommended to understand the effects of physicochemical properties of fiber besides solubility and fermentability to enable the strategic inclusion of ingredients in diets for animals, as well as using TDF values for energy prediction equations.

FIGURE AND TABLES

Figure 3.1. Fiber components included in each analysis for dietary fiber[1]



[1]Adapted from Lancheros et al. (2022).

Table 3.1. Insoluble (IDF), soluble (SDF), and total dietary fiber (TDF) in cereal grains, 88% dry matter (DM) basis

Feed ingredient	N[1]	DM	SD[1]	IDF	SD	SDF	SD	TDF	SD	Calculated TDF[2]
Barley	33	90.21	4.34	16.06	2.01	3.61	0.77	19.67	2.20	23.38
Barley, dehulled	1	89.09	-	9.38	-	3.26	-	12.64	-	16.84
Corn	115	89.09	4.00	10.54	2.17	1.27	2.42	11.81	3.75	12.50
Corn, extruded	7	89.91	1.64	8.63	0.49	1.37	1.19	10.00	1.20	12.50
Corn, high oil	1	89.54	-	15.04	-	0.00	-	15.04	-	12.50
Oats	6	89.17	1.52	31.96	4.32	3.10	0.54	35.06	3.85	30.94
Oats, dehulled	3	88.54	0.45	8.11	2.32	4.28	0.30	12.39	2.34	9.02
Oats, extruded	1	91.20	-	24.77	-	3.90	-	28.67	-	30.94
Rice, brown	2	86.98	0.81	3.29	0.04	0.23	0.10	3.52	0.06	2.21
Rye	4	87.23	2.24	14.16	1.00	4.73	3.15	18.89	4.02	14.41
Rye, hybrid	9	88.23	1.19	13.79	0.81	2.78	0.81	16.57	1.16	14.41
Sorghum	3	88.46	0.62	8.45	1.10	0.23	0.15	8.68	1.07	4.84
Sorghum, high lysine	2	89.28	0.03	10.00	2.44	0.30	0.28	10.30	2.16	12.31

Table 3.1. (cont.)

Sorghum, red	3	88.67	0.03	9.26	1.15	0.26	0.23	9.52	1.38	10.83
Sorghum, red extruded	1	88.70	-	7.64	-	0.30	-	7.94	-	10.83
Sorghum, white	3	88.64	0.05	8.60	1.86	0.23	0.25	8.83	1.94	11.28
Sorghum, white extruded	1	89.12	-	6.42	-	0.59	-	7.01	-	11.28
Wheat	35	89.77	4.98	10.90	1.52	1.18	0.59	12.08	1.76	10.14

[1]N = number of samples analyzed; SD = standard deviation.

[2]Calculated using NRC (2012) and Stein (2025) data as DM - (ash + protein + fat + starch), adjusted to 88% DM.

Table 3.2. Insoluble (IDF), soluble (SDF), and total dietary fiber (TDF) in cereal grain co-products, 88% dry matter (DM) basis

Feed ingredient	N[1]	DM	SD[1]	IDF	SD	SDF	SD	TDF	SD	Calculated TDF[2]
Bakery meal	72	90.74	2.06	15.10	3.44	1.55	1.55	16.65	3.73	17.07
Barley rootlets	5	96.64	0.67	29.15	2.20	1.62	0.62	30.77	1.86	-
Corn bran	3	90.07	0.63	38.46	8.96	2.36	2.40	40.82	9.41	35.99
Corn DDGS[3]	64	87.77	1.74	33.68	3.13	2.39	1.45	36.07	3.16	38.82
Corn DDGS, post-MSC[3]	2	88.64	1.95	36.77	8.04	2.14	0.26	38.91	8.30	38.15
Corn DDGS, high protein	4	88.19	0.60	34.18	1.87	3.01	0.97	37.19	2.12	28.57
Corn fermented protein	5	93.55	0.87	28.50	1.21	1.64	0.91	30.14	1.70	33.82
Corn germ meal	6	90.27	0.97	33.50	5.41	3.08	1.79	36.58	4.65	43.17
Corn gluten feed	4	89.55	1.06	34.41	7.68	2.73	1.63	37.14	6.20	37.09
Corn gluten meal	4	92.90	5.04	6.79	3.83	0.68	0.44	7.47	3.76	7.49
Corn starch	1	89.85	-	0.00	-	0.01	-	0.01	-	2.09
Oat hulls	2	96.20	5.37	66.36	4.61	1.25	0.39	67.61	5.01	74.89
Rice bran, full fat	1	94.85	-	27.09	-	3.80	-	30.90	-	20.10

Table 3.2. (cont.)

Rice bran, defatted	1	89.32	-	21.87	-	1.45	-	23.32	-	32.75
Rice, broken	4	87.72	0.25	1.97	0.46	0.17	0.20	2.28	0.63	0.00
Rice flour	2	87.85	0.21	1.25	0.21	0.00	-	1.25	0.50	0.00
Rice mill feed	1	90.01	-	51.72	-	1.78	-	53.50	-	49.62
Rice protein	1	98.35	-	6.53	-	0.18	-	6.71	-	11.86
Sorghum DDGS	2	90.11	0.00	32.08	4.76	4.10	0.97	36.18	5.80	41.14
Wheat bran	26	91.04	4.53	36.54	3.57	2.45	0.70	38.99	3.61	41.15
Wheat DDGS	3	90.85	1.65	24.91	0.63	3.36	1.43	28.27	1.88	39.56
Wheat flour	3	93.67	5.50	2.65	0.63	1.96	1.40	4.61	2.02	0.00
Wheat gluten feed	2	90.85	0.78	28.42	2.30	2.28	0.75	30.70	3.04	38.56
Wheat middlings	26	89.44	0.73	35.42	4.84	2.60	0.72	38.02	4.82	45.74

[1]N = number of samples analyzed; SD = standard deviation.

[2]Calculated using NRC (2012) and Stein (2025) data as DM - (ash + protein + fat + starch), adjusted to 88% DM.

[3]DDGS = Distillers dried grains with solubles; MSC = Maximized stillage co-products system.

Table 3.3. Insoluble (IDF), soluble (SDF), and total dietary fiber (TDF) in oilseed co-products, 88% dry matter (DM) basis

Feed ingredient	N[1]	DM	SD[1]	IDF	SD	SDF	SD	TDF	SD	Calculated TDF[2]
00-Rapeseed expellers	3	89.25	0.43	32.07	1.89	3.72	0.78	35.76	1.43	37.37
00-Rapeseed expellers fermented	1	89.00	-	26.10	-	2.37	-	28.47	-	-
00-Rapeseed meal	21	89.40	1.17	29.79	2.31	2.37	0.73	32.14	2.33	43.91
Canola expellers	2	91.41	0.05	26.72	0.49	4.51	1.34	31.23	0.85	35.69
Canola meal	8	90.57	1.91	27.72	2.96	3.08	1.78	30.79	3.33	36.28
Copra expellers	4	89.94	4.28	40.85	1.95	4.29	2.02	45.14	3.48	45.37
Copra meal	4	92.29	3.38	38.69	3.61	4.02	0.87	42.71	3.80	51.20
Cottonseed meal	1	90.95	-	26.80	-	3.00	-	29.80	-	36.51
Palm kernel expellers	1	91.90	-	60.90	-	2.60	-	63.50	-	60.41
Palm kernel meal	4	92.30	0.71	58.57	5.06	2.68	0.66	61.26	4.54	62.57
Soy protein concentrate	15	94.97	3.75	17.66	2.95	1.95	1.46	19.61	3.27	17.47
Soy protein isolate	2	94.24	0.64	2.33	1.70	0.47	0.27	2.80	1.43	0.10
Soybean hulls	14	90.45	1.65	62.24	2.27	5.58	1.75	67.82	1.98	68.48

Table 3.3. (cont.)

Soybean hulls, extruded	2	92.64	0.35	60.22	0.23	7.31	0.03	67.53	0.26	-
Soybean meal	145	90.06	2.90	16.01	3.02	2.22	2.25	18.23	3.28	35.07
Soybean meal, enzyme-treated	7	93.15	1.41	15.16	0.95	2.08	1.34	17.24	1.51	24.99
Soybean meal, fermented	12	90.27	4.49	14.78	3.09	2.63	1.38	17.41	3.36	26.19
Soybeans, expellers	7	91.81	2.02	17.23	1.18	3.32	1.54	20.55	1.73	29.85
Soybeans, full-fat	23	95.86	2.79	20.42	3.53	2.46	1.42	22.88	4.03	26.53
Soybeans, full-fat fermented	2	88.7	0.36	16.27	0.07	4.46	0.02	20.73	0.08	-
Sunflower expellers	1	96.18	-	36.87	-	4.12	-	40.99	-	47.15
Sunflower meal	23	90.50	1.48	39.56	6.65	3.85	2.27	43.41	6.39	46.21
Sunflower protein concentrate	1	95.30	-	2.68	-	2.59	-	5.27	-	-

[1]N = number of samples analyzed; SD = standard deviation.

[2]Calculated using NRC (2012) and Stein (2025) data as DM - (ash + protein + fat + starch), adjusted to 88% DM.

Table 3.4. Insoluble (IDF), soluble (SDF), and total dietary fiber (TDF) in other feed ingredients, 88% dry matter (DM) basis

Feed ingredient	N[1]	DM	SD[1]	IDF	SD	SDF	SD	TDF	SD	Calculated TDF[2]
Alfalfa meal	1	84.60	-	49.48	-	2.90	-	52.38	-	56.25
Chicory pulp	1	89.80	-	47.00	-	15.21	-	62.21	-	-
Faba beans	1	88.90	-	16.33	-	1.48	-	17.81	-	16.99
Field peas	12	89.41	1.14	15.68	0.83	1.74	0.40	17.42	1.05	18.39
Flaxseed meal	1	92.90	-	48.26	-	5.23	-	53.49	-	39.08
Lupins	1	88.80	-	43.48	-	2.76	-	46.24	-	40.07
Pea flakes	2	89.30	0.71	15.14	4.09	2.17	2.48	17.31	6.57	-
Pea protein concentrate	2	96.96	4.31	7.57	1.62	1.11	0.82	8.68	2.44	-
Pea starch	5	90.18	2.37	2.20	1.52	0.18	0.25	2.38	1.65	-
Pectin	2	85.28	7.74	0.53	0.62	52.94	5.61	53.47	4.99	54.92
Pistachio	2	96.70	0.91	13.48	1.54	0.96	0.33	14.44	1.87	-
Pistachio blanks	1	93.92	-	69.90	-	6.09	-	75.99	-	76.82
Pistachio shell powder	2	97.29	1.40	77.72	8.86	4.53	4.86	82.25	4.00	84.62

Table 3.4. (cont.)

Potato protein concentrate	11	94.21	4.63	5.62	2.31	0.10	0.15	5.72	2.36	10.01
Sugar beet pulp	25	89.42	3.86	49.11	6.75	14.90	5.23	64.01	7.05	71.15

[1]N = number of samples analyzed; SD = standard deviation.

[2]Calculated using NRC (2012) and Stein (2025) data as DM - (ash + protein + fat + starch), adjusted to 88% DM.

Table 3.5. Means of the difference between calculated and analyzed total dietary fiber (TDF) within and among feed ingredient groups.

	Cereal grains	Cereal grain co-products	Oilseed co-products	Other feed ingredients	SEM[1]	*P*-value
Delta TDF	-0.29[b]	1.43[ab]	4.57[a]	-1.10[b]	1.34	0.017
SEM[1]	0.75	1.20	1.20	2.05		
P-value	0.702	0.249	0.001	0.603		

[1]SEM = standard error of the mean.

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CHAPTER 4: TOLERANCE OF WEANLING PIGS AND EFFECTS ON GROWTH PERFORMANCE OF SUPPLEMENTING CORN-SOYBEAN MEAL-BASED DIETS WITH GRADED LEVELS OF A NOVEL EXOGENOUS B-MANNANASE

ABSTRACT

The hypothesis that a novel endo-β-mannanase can be used in diets for pigs for a period of 42 days post-weaning without negatively impacting growth performance, serum chemistry, hematological characters, or organ weights was tested. A total of 150 newly weaned pigs (75 castrated male and 75 female pigs; initial body weight: 6.20 ± 0.68 kg) were used. Pigs were allotted to three experimental diets (i.e., control, control plus 800 thermostable mannanase units (**TMU**)/kg, or control plus 100,000 TMU/kg). Pigs were allotted to pens with 5 pigs per pen for a total of 10 replicate pens per treatment. Pigs were fed phase 1 diets from d 1 to 21, and phase 2 diets from d 22 to 42 post-weaning. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed (**G:F**) were calculated. Blood samples from two pigs per pen (one male and one female pig) were collected on d 1, 21, and 42. One pig per pen from the control treatment and two pigs per pen from each of the β-mannanase treatments were euthanized at the end of the experiment and organs were collected. Data were analyzed using the proc MIXED procedure of SAS with pen as the experimental unit. Results indicated that for the overall experiment, there were no differences in ADG, ADFI, or final body weight among treatments. However, pigs fed the diet with 100,000 TMU/kg of β-mannanase had greater ($P < 0.05$) G:F from d 22 to 42 and for the overall experimental period compared with pigs fed the control diet or the diet with 800 TMU/kg of β-mannanase. Most serum chemistry markers and blood hematological characters were not different among pigs fed experimental diets and

concentrations were within the normal biological range for pigs. However, serum phosphorus was greater ($P < 0.05$) in pigs fed the diet with 100,000 TMU/kg of β-mannanase compared with pigs fed the other diets, but red cell distribution width and mean platelet volume were greater ($P < 0.05$) in pigs fed the control diet compared with pigs fed the control diet + 800 TMU/kg of β-mannanase. Abnormalities in liver, kidney, spleen, heart, stomach, or the small intestine were not observed, and the weight of these organs was not affected by dietary treatments. In conclusion, pigs fed diets containing 100,000 TMU/kg of β-mannanase had greater G:F from d 1 to 42 post-weaning compared with pigs fed control diets or the diets with 800 TMU/kg, and β-mannanase did not negatively impact general health and growth of the pigs even if included at a very high dose.

Keywords: growth performance, tolerance, weanling pigs, β-mannanase.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ALKP, alkaline phosphatase; AST, aspartate transaminase; BUN, blood urea nitrogen; EDTA, ethylenediaminetetraacetic acid; G:F, gain to feed ratio; GGT, gamma-glutamyl transferase; IDF, insoluble dietary fiber; LUC, large unstained cells; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cells; RDW, red cell distribution width; SDF, soluble dietary fiber; TDF, total dietary fiber; TMU, thermostable mannanase unit; WBC, white blood cells.

INTRODUCTION

β-mannans are plant cell wall polysaccharides of D-mannose units linked by β-(1-4) glycosidic bonds (Lee and Brown, 2022). β-mannans are a complex carbohydrate and may consist of a long chain of only mannose units or a chain of mannose units with side chains of α-1,6-linked galactose or glucose residues, resulting in galactomannans or galactoglucomannans, respectively (Chen et al., 2018). Soybean meal is a common plant protein in diets for pigs due to its well-balanced amino acid profile and digestibility. However, soybean meal also contains anti-nutritional factors such as allergenic proteins and non-starch polysaccharides, which limits its use in diets for weanling pigs (Koepke et al., 2017). Soybean meal contains 17 to 27% non-starch polysaccharides, including 0.7 to 2.1% β-mannans (Bach Knudsen, 1997; Hsiao et al., 2006; Kiarie et al., 2021). β-mannans in diets for pigs cannot be hydrolyzed because pigs lack the endogenous enzymes that target the β-1-4-mannosyl bonds. As a result, β-mannans pass through the small intestine undigested, but they may reduce water absorption by increasing digesta viscosity and water excretion through the feces, due to their solubility and high water-holding capacity that causes impaired diffusion of digestive enzymes, resulting in reduced digestibility of nutrients (Jang et al., 2020; Kiarie et al., 2021). Pigs also experience high stress at weaning, resulting in significant physiological and immunological changes, including reduced feed intake, impaired intestinal function, and increased susceptibility to diseases (Campbell et al., 2013). However, supplementation of an exogenous β-mannanase to diets for weanling pigs may mitigate the negative impacts of β-mannans on pig growth and immune response (Lee and Brown, 2022; Baker et al., 2024). Recently a novel endo-β-mannanase, Natupulse® TS, was developed, but there are no data demonstrating the efficiency, the safety, or the tolerance to an overdose of this enzyme when included in diets for weanling pigs. Safety of feed enzymes needs

to be determined to avoid undesirable effects on the target animal, such as allergies and irritations (Pariza and Cook, 2010). Therefore, it was hypothesized that if pigs can tolerate a very high dose of the enzyme (i.e., 100 times the recommended inclusion level), the β-mannanase can be considered safe and will not cause undesirable effects if included in diets for pigs. Therefore, an experiment was conducted to test the hypothesis that the novel β-mannanase can be added to corn-soybean meal diets fed to weanling pigs during the initial 42 days post-weaning without negative effects on growth performance or health, even if included at a very high dose.

MATERIALS AND METHODS

The protocol for the experiment was submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Pigs were the offspring of Line 800 males mated to Camborough females (Pig Improvement Company, Henderson, TN, USA).

Animals, housing, and experimental design

A total of 150 newly weaned pigs (75 castrated male and 75 female pigs; initial body weight: 6.20 ± 0.68 kg) were allotted to one of three experimental diets using a randomized complete block design, with weaning weight as the blocking factor. Gender was balanced within each pen and across treatments. Thus, within each treatment, there were 5 pens with 3 barrows and 2 gilts, and 5 pens with 2 barrows and 3 gilts for a total of 10 replicate pens per treatment.

A 2-phase feeding program was used with d 1 to 21 as phase 1, and d 22 to 42 as phase 2. In each phase, three diets based on corn and soybean meal were formulated to contain 0 thermostable mannanase units (**TMU**)/kg (control diet), 800 TMU/kg, or 100,000 TMU/kg, respectively (Tables 4.1, 4.2, and 4.3). The mannanase unit TMU is defined as the amount of

enzyme that produces reducing carbohydrates that have a reducing power corresponding to one μmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5. The β-mannanase (*Natupulse® TS*) was supplied by BASF SE, Ludwigshafen, Germany. The 0, 800, and 100,000 TMU/kg feed activity levels were equivalent to 0, 100, and 12,500 mg of β-mannanase per kg of diet, respectively, and the β-mannanase was included in the diets at the expense of corn. The recommended inclusion in corn-soybean meal diets of this β-mannanase in 800 TMU/kg and the 100,000 TMU/kg inclusion was tested to determine if an overdose of β-mannanase has negative impacts on pig growth or health. All dietary nutrients were included in the diets to meet or exceed current requirement estimates (NRC, 2012). All diets were fed in mash form. Throughout the experiment, pigs had free access to feed and water.

Pigs were housed in floor pens (1.2 × 1.4 m) in an environmentally controlled barn. Floors were fully slatted with plastic coating. A 4-hole feeder and a nipple drinker were installed in each pen. Temperature, humidity, lightning, feeder and water space were identical for all experimental groups. Barns had a negative pressure ventilation system and had lights turned on at all times. Barn temperatures were 30 ºC in week 1 post-weaning, 28 ºC in week 2, 26 ºC in week 3, 24 ºC in week 4, and 22 ºC in weeks 5 and 6 post-weaning.

Pigs received routine vaccinations before the start of the experiment, but were not vaccinated during the experiment. There was no routine application of medications during the experiment. General health status, morbidity, and mortality were recorded twice daily.

Blood sample collection and chemical analyses

Two samples of blood were collected on d 1, 21, and 42 from the jugular vein of two pigs per pen (one male and one female pig). Within each pen, the same pig was bled on the three

sampling days. For the first sample, approximately 6 mL of whole blood was collected into a serum separation vacutainer. Blood was allowed to clot for 15 to 30 minutes before centrifuging at 769 × g per 10 min to yield blood serum, which was transferred into sterile microtubes. For the second sample, approximately 5 mL of whole blood was collected into a vacutainer containing ethylenediaminetetraacetic acid (**EDTA**). Immediately after collection, tubes were gently inverted several times to ensure thorough mixing of the blood and anticoagulant. Serum and blood EDTA tubes were shipped on ice packs right after collection to the Clinical Pathology Laboratory at Iowa State University, Ames, IA, USA, for analysis. Serum samples were analyzed for chemistry markers, including sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, blood urea nitrogen (**BUN**), creatinine, glucose, total protein, albumin, aspartate transaminase (**AST**), creatine kinase, alkaline phosphatase (**ALKP**), gamma-glutamyl transferase (**GGT**), total bilirubin, and anion gap. Blood EDTA samples were analyzed for hematology profile including total white blood cells count (**WBC**), WBC differential (i.e., neutrophil, lymphocyte, monocyte, eosinophil, basophil), absolute large unstained cells (**LUC**), red blood cell count (**RBC**), hemoglobin, hematocrit, mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), mean corpuscular hemoglobin concentration (**MCHC**), red cell distribution width (**RDW**), platelet count, and mean platelet volume (**MPV**).

Ingredients and diets were analyzed for dry matter (Method 930.15; AOAC Int., 2019) and nitrogen was analyzed using the combustion procedure (Method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as nitrogen × 6.25. Diets and ingredients were also analyzed for ash (Method 942.05; AOAC Int., 2019) and insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) were analyzed according to method 991.43 (AOAC Int., 2019) using the Ankom TDF Dietary Fiber Analyzer (Ankom

Technology, Macedon, NY, USA). Total dietary fiber (**TDF**) was calculated as the sum of IDF and SDF. Gross energy in diets and ingredients was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Diets and ingredients were analyzed for acid hydrolyzed ether extract by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Method 2003.06; AOAC Int., 2019] using petroleum ether (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Diets were analyzed for amino acids [Method 982.30 E (a, b, c); AOAC Int., 2019] on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for post column derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 hours at 110 ºC [Method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [Method 982.30 E(b); AOAC Int., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 hours at 110 ºC [method 982.30 E(c); AOAC Int., 2019]. Diets were analyzed for β-mannanase activity using procedure FEN/0005/01 (BASF SE, Ludwigshafen, Germany). Soybean meal was also analyzed for mannose using gas-liquid chromatography based on the individual sugar constituent as alditol acetates (Oxley et al., 2004).

Necropsy and histopathology examinations

One pig per pen from the control treatment and two pigs per pen from the β-mannanase treatments were euthanized at the end of the experiment on d 42. The liver, kidney, spleen, heart, stomach, and small intestine from these pigs were examined by a board-certified pathologist for visual abnormalities or indications of toxicity. The weights of these organs were also recorded.

Calculations and statistical analyses

Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each phase. Daily feed allotments were recorded, and feed left in the feeders was weighed at the end of each phase to calculate feed disappearance. If a pig was removed from a pen during the experiment, the feed left in the feeder and individual weights of the remaining pigs in the pen were recorded on the day the pig was removed. Feed intake for the remaining pigs in the pen was adjusted for the feed consumed by the pig that was removed based on weight gain (Lindemann and Kim, 2007; Lee et al., 2016). Data were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**) and average gain to feed ratio (**G:F**) with the pen as the experimental unit. Data were calculated for phase 1 (d 1 to 21), phase 2 (d 22 to 42) and for the overall experiment (d 1 to 42).

No outliers were removed from the dataset. Growth performance data were analyzed using the PROC MIXED procedure of SAS (SAS Stats Inc. Cary, NC, USA). Because blood samples were collected on day 1, 21, and 42 from the same pigs, an additional analysis was conducted to analyze the effect of time on serum chemistry and hematology markers. These data were analyzed as repeated measures with unstructured variance using the MIXED and REPEATED procedures of SAS. For serum chemistry and hematology markers, the average of the two pigs represented the pen, and the pen was the experimental unit for all analysis. The statistical model included diet as fixed effect and replicate as random effect. Mean values were calculated using the LSMeans statement and if significant differences were identified, means were separated using the PDIFF procedure with Tukey adjustment (Tukey, 1977). Statistical significance was considered at $P < 0.05$.

RESULTS

Growth performance

There were no differences in initial body weight, ADG, ADFI, or G:F of pigs from d 1 to 21, or in the body weight of pigs on d 21 (Table 4.4). There were also no differences in ADG and ADFI of pigs from d 22 to 42 but pigs fed the control diet + 100,000 TMU/kg of β-mannanase had greater ($P < 0.05$) G:F compared with pigs fed the control diet or the control diet + 800 TMU/kg of β-mannanase from d 22 to 42. For the entire experimental period (d 1 to 42) no differences in ADG, ADFI, or final body weight were observed among treatments, but G:F was greater for pigs fed the control diet + 100,000 TMU/kg of β-mannanase compared with pigs fed the control diet or the control diet + 800 TMU/kg of β-mannanase. The mortality was 2% (n = 1) for pigs fed the control diet or the control diet + 100,000 TMU/kg of β-mannanase, and 4% (n = 2) for pigs fed the control diet + 800 TMU/kg of β-mannanase; however, the difference is not significant.

Serum chemistry markers

There were no differences in sodium, potassium, chloride, bicarbonate, calcium, magnesium, BUN, creatinine, glucose, total protein, albumin, AST, creatine kinase, ALKP, GGT, or total bilirubin among experimental diets (Table 4.5). However, phosphorus was greater ($P < 0.05$) in pigs fed the control diet + 100,000 TMU/kg of β-mannanase compared with pigs fed the control diet or the control diet + 800 TMU/kg of β-mannanase.

Hematological characters

There were no differences in WBC, RBC, hemoglobin, hematocrit, MCV, MHC, MCHC, platelet, neutrophil, lymphocyte, monocyte, eosinophil, and basophils among experimental diets (Table 4.6). However, RDW and MPV were greater ($P < 0.05$) in pigs fed the control diet

compared with pigs fed the control diet + 800 TMU/kg of β-mannanase, but pigs fed the diets with 100,000 TMU/kg of β-mannanase were not different from pigs fed the control diets.

Necropsies and organ weights

Necropsies conducted at the end of the experiment revealed no lesions in organs of pigs fed the control diet + 800 TMU/kg of β-mannanase or in organs of pigs fed the control diet + 100,000 TMU/kg of β-mannanase. There were also no differences on d 42 post-weaning in the weights of organs among pigs fed the three experimental diets (Table 4.7).

DISCUSSION

Concentrations of dry matter, gross energy, ash, acid-hydrolyzed ether extract, IDF, SDF, and TDF, and crude protein in ingredients were in agreement with reported values (NRC, 2012). Likewise, the analyzed nutrient composition of the diets and the β-mannanase activity in the diets used in the experiment were in agreement with calculated values.

β-mannans are linear polysaccharides formed from repeating β-(1-4) mannose units and are part of the cell wall in leguminous plants (Jackson et al., 2004; Lee and Brown, 2022). The β-mannan backbone may have sidechains containing galactose or glucose monomers that are linked to the backbone via α-(1-6) glycosidic bonds. Soybean meal contains between 0.7% and 2.1% β-mannans, which are mostly associated with the hull fraction (Kiarie et al., 2021); therefore, it was expected that the soybean meal used in this experiment provided the substrate for the enzyme tested. To estimate the content of β-mannan in soybean meal, the monosaccharide mannose concentration was analyzed. However, the analyzed mannose in the soybean meal used in this experiment was low compared with reported values (Hsiao et al., 2006), indicating that the content of β-mannans in the soybean meal used in this experiment is also low, possibly due

to the removal of the hulls during soybean processing because dehulled soybean meal contain less β-mannan than soybean meal with hulls (Hsiao et al., 2006).

The lack of an effect of β-mannanase on ADG was in agreement with data from experiments using β-mannanase in diets for weaning pigs (Huntley et al., 2018; Jang et al., 2020; 2024). Likewise, the improved G:F in response to the addition of β-mannanase to the diets is in agreement with previous data (Kiarie et al., 2021; Baker et al., 2024; Tajudeen et al., 2025). Addition of β-mannanase to diets for weanling pigs may increase hydrolysis of the backbone of galactomannans resulting in generation of manno-oligosaccharides, which may be fermented by microbial enzymes in the hindgut (Pettey et al., 2002). Therefore, β-mannanase may increase fermentability of IDF, increases the digestible energy of the diet, and results in greater G:F (Pettey et al., 2002; Kiarie et al., 2013). Likewise, β-mannanase may decrease the water holding capacity of β-mannans and decrease digesta viscosity in the small intestine, as has been demonstrated in poultry (Jackson et al., 2004; Chegeni et al., 2011; Fickler et al., 2023) and weanling pigs (Baker et al., 2024; Jang et al., 2024), resulting in an increase in the activity and efficiency of digestive enzymes to reach the substrates and consequently improve nutrient and energy digestibility. Differences in effects of β-mannanase among recent experiments may be due to differences in the amount of β-mannans in the diets, indicating that if a relatively small amount of manno-oligosaccarides are released from β-mannan hydrolysis by the β-mannanase enzyme, a low or non-detectable energy contribution for the animals may be the result, which is the reason for a lack of an effect on ADG. However, the β-mannanase effect on G:F may be a result of increased growth rate and not increased feed intake (Kiarie et al., 2021), which would indicate a greater energy digestibility. Because nutrient digestibility and digesta viscosity were

not determined in this experiment, this hypothesis cannot be verified, but warrants further research.

Serum chemistry markers provide insight into potential disruptions in organ activity. For example, BUN, AST, and creatinine indicate dysregulation of skeletal or cardiac muscle, whereas bilirubin, glucose, calcium, phosphorus, ALKP, albumin, total protein, GGT, and AST provide information about changes in liver function (Wilson et al., 1972; Rymut et al., 2021). Likewise, glucose and triglycerides provide information about energy balance, and chlorine, sodium, potassium, and bicarbonate are indicators of disorders in the metabolic system and digestive function, whereas anion gap indicates the difference between positively and negatively charged electrolytes (Kaneko et al., 1997; Rymut et al., 2021). The observation that with the exception of the concentration of phosphorus none of the serum chemistry markers were different among treatments indicates that regardless of treatment, pigs were healthy throughout the experiment, and concentrations of all serum markers were within the normal biological range for pigs (Iowa State University Clinical Pathology Laboratory, 2011; Cooper et al., 2014). This was also true for pigs fed the diets containing 100,000 TMU of β-mannanase, demonstrating that even when the dose of the enzyme is higher than the recommended inclusion rate, no negative impact on pig health was observed.

Exogenous enzymes are generally not considered toxic when added to diets for pigs due to their substrate specificity and catalytic activity (Lessard et al., 2021). The observation that the β-mannanase used in this experiment did not result in increased mortality of pigs is in agreement with other experiments using β-mannanase fed to pigs (Sánchez-Uribe et al., 2022; Tajudeen et al., 2025), indicating that the enzyme is safe. Likewise, the observation that the majority of hematology characters and blood chemistry markers, which are sensitive to nutritional

malabsorption, disease, and other physiological disorders, were not influenced by supplementation of β-mannanase in the diets, indicates that the physiology of the animals was not altered by consuming the enzyme even at a high inclusion level. However, the observation that supplementation of β-mannanase did not influence the majority of serum chemistry markers 42-days post-weaning, is in contrast with data indicating increased blood glucose concentrations when β-mannanase was included in diets for growing pigs (Kim et al., 2013; 2017). The increased glucose in serum may be explained by greater glucose absorption after the hydrolysis of glucomannans. However, the β-mannans in the diets used in this experiment are expected to be galactomannans, because they are present in legumes, such as soybeans (de Vries and Visser, 2001), which may be the reason that glucose absorption was not increased in this experiment. Serum concentrations differences of phosphorus and blood RDW and WBC did not indicate a negative impact of the enzyme. The lack of differences in serum and blood parameters has been previously documented in porcine animals fed diets containing exogenous enzymes (Schliffka et al., 2019; Lessard et al., 2021).

The observation that supplementation with β-mannanase to diets for weanling pigs did not influence the weight of the organs or generate pathological lesions is in agreement with results of research demonstrating that there are no changes in the weight of organs of pigs fed a multienzyme supplement compared with pigs fed a control diet (Agyekum et al., 2012). The weights of liver, heart, and kidney were in good agreement with weights previously reported for weanling pigs (Choi et al., 2021). Although diets for pigs with high levels of dietary fiber may increase visceral organ weight and intestinal mass, which results in less absorption of nutrients and greater utilization of dietary energy and amino acids for maintenance (Pond et al., 1989), the diets used in this experiment contained a relatively small amount of dietary fiber. Therefore, the

mannooligosaccharides released from β-mannan hydrolysis by the β-mannanase enzyme may not have been able to impact the weight of the organs, which is the reason for a lack of an effect on the weight of the organs after 42 days of feeding.

CONCLUSIONS

The endo-β-mannanase, Natupulse® TS, added to corn-soybean meal diets, improved the G:F of weanling pigs and can be used in diets for pigs without negatively impacting the general health of the pigs. The enzyme had no detrimental effects on serum biochemical and hematological parameters, pathological lesions, organ weight, or growth performance when fed to weanling pigs at the recommended level of 800 TMU/kg or at a tolerance level of 100,000 TMU/kg in diets for pigs during the initial 42 days post-weaning. It is, therefore, concluded that the tested enzyme is safe to use.

TABLES

Table 4.1. Nutrient composition of ingredients, as-is basis

Item	Corn	Soybean meal	Whey powder	Blood plasma
Gross energy, kcal/kg	3,921	4,289	3,637	4,947
Dry matter, %	87.09	89.26	90.74	91.97
Ash, %	1.26	6.06	7.96	6.91
Crude protein, %	6.48	44.45	10.08	80.59
Acid hydrolyzed ether extract, %	3.85	2.26	0.84	0.21
Total dietary fiber, %	9.00	17.35	-	-
Insoluble dietary fiber, %	8.60	15.75	-	-
Soluble dietary fiber, %	0.40	1.60	-	-
Mannose, %	-	0.28	-	-

Table 4.2. Ingredient composition of experimental diets

	Phase 1			Phase 2		
Feedstuff, %	Control	Control + 800 TMU/kg β-Mannanase[1]	Control + 100,000 TMU/kg β-Mannanase	Control	Control + 800 TMU/kg β-Mannanase	Control + 100,000 TMU/kg β-Mannanase
Ground corn	41.90	41.892	40.65	62.63	62.622	61.38
Soybean meal, dehulled	28.00	28.00	28.00	32.00	32.00	32.00
Blood plasma	2.00	2.00	2.00	-	-	-
Choice white grease	1.80	1.80	2.05	2.00	2.00	2.25
Whey powder	23.00	23.00	23.00	-	-	-
L-Lys HCl	0.35	0.35	0.35	0.35	0.35	0.35
DL-Met	0.17	0.17	0.17	0.12	0.12	0.12
L-Thr	0.08	0.08	0.08	0.10	0.10	0.10
Dicalcium phosphate	0.95	0.95	0.95	1.15	1.15	1.15
Limestone	0.85	0.85	0.85	0.75	0.75	0.75
Vitamin-mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50

Table 4.2. (cont.)

Salt	0.40	0.40	0.40	0.40	0.40	0.40
Natupulse TS[3]	0.00	0.008	1.00	0.00	0.008	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[3]The 0, 0.008, and 1.0 inclusion levels are equivalent to 0, 100, and 12,500 mg of β-mannanase per kg of complete diet, respectively.

Table 4.3. Nutrient composition of experimental diets, as-is basis

	Phase 1			Phase 2		
Item	Control	Control + 800 TMU/kg β-Mannanase[1]	Control + 100,000 TMU/kg β-Mannanase	Control	Control + 800 TMU/kg β-Mannanase	Control + 100,000 TMU/kg β-Mannanase
Gross energy, kcal/kg	3,952	3,986	3,999	4,017	4,029	4,045
Dry matter, %	89.01	89.03	89.07	90.74	90.63	90.76
Ash, %	6.02	6.02	5.48	5.09	5.30	4.93
Crude protein, %	18.39	18.59	18.37	18.70	19.14	19.19
Acid hydrolyzed ether extract, %	4.34	4.00	4.68	5.40	5.45	5.32
Total dietary fiber, %	10.05	9.70	10.00	13.45	13.80	13.80
Insoluble dietary fiber, %	8.50	8.40	8.60	12.50	12.50	12.50
Soluble dietary fiber, %	1.55	1.30	1.40	0.95	1.30	1.30
β-mannanase activity, TMU/kg	< 100	961	116,926	< 100	842	115,013
Indispensable AA, %						
Arg	1.18	1.19	1.17	1.27	1.30	1.32

Table 4.3. (cont.)

His	0.51	0.51	0.50	0.51	0.53	0.54
Ile	0.95	0.95	0.92	0.87	0.90	0.92
Leu	1.78	1.79	1.75	1.71	1.73	1.73
Lys	1.48	1.49	1.46	1.38	1.39	1.39
Met	0.45	0.43	0.43	0.38	0.39	0.37
Phe	0.98	0.99	0.96	1.00	1.02	1.05
Thr	0.91	0.92	0.95	0.82	0.83	0.82
Trp	0.27	0.28	0.27	0.23	0.23	0.23
Val	1.05	1.05	1.03	0.98	0.99	1.01
Dispensable AA, %						
Ala	1.05	0.99	0.97	0.99	0.99	1.01
Asp	2.24	2.07	1.97	1.99	1.95	2.04
Cys	0.38	0.37	0.32	0.31	0.30	0.31
Glu	3.80	3.57	3.43	3.55	3.54	3.64
Gly	0.82	0.76	0.74	0.81	0.80	0.83

Table 4.3. (cont.)

Pro	1.18	1.11	1.10	1.13	1.13	1.13
Ser	0.92	0.88	0.86	0.85	0.85	0.87
Tyr	0.68	0.65	0.61	0.64	0.61	0.65

[1]β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

Table 4.4. Growth performance for pigs fed experimental diets[1]

Item	Control	Control + 800 TMU/kg β-Mannanase[2]	Control + 100,000 TMU/kg β-Mannanase	SEM	*P*-value
Phase 1 (d 1 to 21)					
Initial body weight, kg	6.19	6.21	6.19	0.22	0.197
ADG[3], g	200.94	214.10	220.5	9.53	0.349
ADFI[3], g	290.04	305.58	299.73	12.86	0.641
G:F[3]	0.70	0.70	0.74	0.02	0.197
Body weight on d 21, kg	10.41	10.71	10.82	0.33	0.346
Phase 2 (d 22 to 42)					
ADG, g	676.94	663.77	687.61	16.03	0.405
ADFI, g	1,026.17	1,014.28	1,007.11	27.31	0.816
G:F	0.66[b]	0.66[b]	0.68[a]	0.01	0.039
Final body weight, kg	24.63	24.65	25.26	0.62	0.427
Overall Phase (d 1 to 42)					
ADG, g	438.94	438.93	454.05	10.97	0.422
ADFI, g	658.11	659.93	653.42	18.73	0.952
G:F	0.67[b]	0.67[b]	0.70[a]	0.01	0.015
Mortality, %	2.00	4.00	2.00	2.24	0.387

[a-b]Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least square means of 10 observations for all treatments.

Table 4.4. (cont.)

[2]β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

[3]ADG = average daily gain; ADFI= average daily feed intake; G:F = gain-to-feed ratio.

Table 4.5. Serum chemistry markers of pigs fed experimental diets[1]

Item	Reference value[2]	Control	Control + 800 TMU/kg β-Mannanase[3]	Control + 100,000 TMU/kg β-Mannanase	SEM	*P*-value
Sodium, mEq/L	135 – 150	136.37	135.93	135.97	0.28	0.386
Potassium, mEq/L	4 – 7	5.06	4.96	5.11	0.11	0.362
Chloride, mEq/L	95 – 110	100.97	100.53	100.75	0.37	0.627
Bicarbonate, mEq/L	19 - 31	26.72	27.15	26.83	0.48	0.791
Calcium, mg/dl	8 – 12	10.98	10.82	10.81	0.06	0.103
Phosphorus, mg/dl	4.5 – 11.5	10.40[b]	10.42[b]	11.10[a]	0.18	0.017
Magnesium, mg/dl	1.82 – 3.65	2.06	2.04	2.10	0.03	0.133
BUN[4], mg/dl	6 – 30	4.80	4.70	4.97	0.28	0.768
Creatinine, mg/dl	0.5 – 2.7	0.91	0.92	0.94	0.02	0.651
Glucose, mg/dl	65 – 150	119.83	116.32	117.27	1.55	0.367
Total protein, gm/dl	7.0 – 8.9	4.92	4.88	4.91	0.05	0.866

Table 4.5. (cont.)

Albumin, gm/dl	3.0 – 4.5	3.22	3.15	3.16	0.04	0.352
AST[4], IU/L	10 – 300	46.95	49.80	47.02	3.16	0.766
Creatine kinase, IU/L	100 – 2500	898.47	1,029.55	1,018.38	161.56	0.695
ALKP[4], IU/L	25 – 130	570.48	499.87	555.98	31.44	0.352
GGT[4], IU/L	10 – 100	41.62	37.08	34.18	1.57	0.110
Total bilirubin, mg/dl	0 – 1	0.42	0.41	0.42	0.03	0.992
Anion gap	14 - 29	13.76	13.13	13.68	0.47	0.408

[a-b]Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least square means of 10 observations for all treatments.

[2]Reference Intervals were reported by Iowa State University's Clinical Pathology Laboratory (2011) and Cooper et al. (2014).

[3]β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

[4] BUN = blood urea nitrogen; AST = aspartate transaminase; ALKP = alkaline phosphatase; GGT = gamma-glutamyl transferase.

Table 4.6. Hematological characters of pigs fed experimental diets[1]

Item	Reference value[2]	Control	Control + 800 TMU/kg β-Mannanase[3]	Control + 100,000 TMU/kg β-Mannanase	SEM	*P*-value
WBC[4], $\times 10^3$/ul	11.35 – 28.9	15.05	16.50	17.04	0.65	0.105
RBC[4], $\times 10^6$/ul	5.88 – 8.19	6.17	6.19	6.22	0.08	0.929
Hemoglobin, g/dl	11.2 – 14.7	11.13	11.50	11.43	0.15	0.181
Hematocrit, %	32.3 – 42.6	37.14	38.11	38.02	0.45	0.251
MCV[4], fl	47.5 – 59.2	60.26	61.56	61.36	0.74	0.383
MCH[4], pg	16.3 – 20.6	18.06	18.58	18.44	0.26	0.221
MCHC[4], g/dl	33.3 – 35.8	29.96	30.18	30.08	0.12	0.389
RDW[4], %	16.4 – 32.3	20.85[a]	19.15[b]	19.47[ab]	0.47	0.031
Platelet, $\times 10^3$/ul	118.9 – 522.9	388.88	402.46	411.08	22.50	0.780
MPV[4], fl	6.8 – 10.8	10.61[a]	9.67[b]	9.94[ab]	0.23	0.028
Neutrophil, $\times 10^3$/ul	2.0 – 10.4	6.92	8.02	8.04	0.59	0.315

Table 4.6. (cont.)

Lymphocyte, $\times 10^3$/ul	5.30 – 17.9	7.07	7.39	7.75	0.27	0.169
Monocyte, $\times 10^3$/ul	0.0 – 3.7	0.55	0.55	0.62	0.03	0.170
Eosinophil, $\times 10^3$/ul	0.0 – 1.3	0.31	0.33	0.36	0.04	0.574
Basophils, $\times 10^3$/ul	0.0 – 0.4	0.05	0.06	0.06	0.01	0.625
Absolute LUC[4], $\times 10^3$/ul	-	0.13	0.16	0.16	0.01	0.269

[a-b]Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least square means of at least 8 observations for all treatments.

[2]Reference Intervals were reported by Iowa State University's Clinical Pathology Laboratory (2011) and Cooper et al. (2014).

[3]β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

[4]WBC = total white blood cells count; RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume; LUC = absolute large unstained cells.

Table 4.7. Weights of organs of pigs at necropsy on d 42 post-weaning[1, 2]

Item	Control	Control + 800 TMU/kg β-Mannanase[3]	Control + 100,000 TMU/kg β-Mannanase	SEM	*P*-value
Liver, g	895	865	875	35	0.794
Kidney, g	148	150	143	6	0.445
Spleen, g	58	54	54	3	0.658
Heart, g	153	155	147	6	0.297
Stomach, g	705	673	683	41	0.869
Small intestine, g	1,725	1,650	1,695	53	0.555

[1]Data are least squares means for each dependent variable represent 10 observations for the control treatment and 20 observations for the control + 800 TMU/kg and control + 100,000 TMU/kg treatments.

[2]All organs were inspected for pathological changes, but no abnormalities were detected.

[3]β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

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CHAPTER 5: EVALUATION OF EFFICACY OF A NOVEL XYLANASE AND B-GLUCANASE COMBINATION ON ENERGY AND TOTAL DIETARY FIBER DIGESTIBILITY IN GROWING PIGS

ABSTRACT

The objective of this experiment was to test the hypothesis that supplementation of a novel combination of xylanase and β-glucanase in diets for growing pigs will increase the apparent total tract digestibility (**ATTD**) of gross energy and total dietary fiber (**TDF**), and therefore, also increase metabolizable energy (**ME**). Seventy-two barrows (initial body weight: 27.83 ± 2.16 kg) were allotted to three diets with 24 replicate pigs per diet in a randomized complete block design with 3 blocks of 24 pigs. A control diet containing wheat, barley, soybean meal, canola meal, and wheat middlings was formulated. Two additional diets were formulated by supplementing either a low or high dose of the enzyme combination to the control diet (xylanase and β-glucanase, LUNA XB101; Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands). Pigs were housed individually in metabolism crates and fed experimental diets for 15 days, with fecal and urine samples collected for 5 days to determine ATTD of dry matter, gross energy, insoluble dietary fiber, soluble dietary fiber, and TDF, as well as digestible energy (DE) and ME. Results indicated that pigs fed the high enzyme dose tended to have greater ($P < 0.10$) ATTD of gross energy and DE, and had greater ($P < 0.05$) ME compared with pigs fed the control diet. However, no differences were observed in ATTD of dry matter, insoluble dietary fiber, soluble dietary fiber, or TDF. These results indicated that the enzyme combination may improve utilization of energy by pigs fed high-fiber diets, likely through partial hydrolysis of arabinoxylans and β-glucans, which may have increased microbial fermentation in the hindgut,

resulting in more absorption of short-chain fatty acids. In conclusion, supplementation with the novel xylanase and β-glucanase combination may increase utilization of energy in fiber rich diets by growing pigs, but more research is needed to understand its effects to increase digestibility of fiber.

Keywords: β-glucanase, digestibility, energy, fiber, pigs, xylanase.

Abbreviations: ATTD, apparent total tract digestibility; BGU**,** β-glucanase units; DE, digestible energy; IDF, insoluble dietary fiber; ME, metabolizable energy; NSP, non-starch polysaccharides; SDF, soluble dietary fiber; TDF, total dietary fiber; XU, xylanase units.

INTRODUCTION

The feed industry is increasing the use of co-products as from the human food industry, which often results in greater inclusion of dietary fiber in diets for pigs (Woyengo et al., 2014). Dietary fiber is a part of the carbohydrate portion of plant-based ingredients used in diets for pigs. Dietary fiber includes non-starch polysaccharides (**NSP**), such as cellulose and non-cellulosic polysaccharides, and lignin, and is considered undigestible because pigs lack endogenous enzymes needed to hydrolyze the glycosidic bonds between monosaccharides forming dietary fiber structures (Choct, 2015). However, some of the fiber may be fermented in the hindgut by intestinal microbes who can express enzymes needed for hydrolysis of NSP (Bach Knudsen et al., 2012; Navarro et al., 2019). Despite microbial activity, dietary fiber is only partially hydrolyzed because the microbial populations in the hindgut do not express all the enzymes needed for digestion of all glycosidic bonds in fiber (Stein, 2019). Greater inclusion of dietary fiber in diets for pigs and low or partial fermentation may reduce nutrient availability

because dietary fiber may trap nutrients and increase the viscosity of digesta, which may impair the ability of digestive enzymes to reach their substrate, resulting in reduced digestibility and absorption of nutrients (Jha et al., 2010; Gutierrez et al., 2016).

To reduce the negative effects of fiber on small intestinal nutrient digestion and to increase fiber fermentation in the hindgut, exogenous carbohydrases may be added to diets for pigs, individually or in combinations, to hydrolyze specific glycosidic bonds in the fiber fraction. Xylanase hydrolyzes the β-1,4 glycosidic bonds present in the backbone of arabinoxylans, the main NSP in cereal grains, which results in the release of xylo-oligosaccharides (Jaworski et al., 2015; Navarro et al., 2019). Endo 1,3 β-glucanase hydrolyzes the 1,3-glycosidic bonds of mixed-linked β-glucans, primarily present in barley and oats, which results in release of gluco-oligosaccharides (Caseiro et al., 2022). Both xylo- and gluco-oligosaccharides can act as a substrate for microbes, resulting in an increase in the abundance of specific bacteria and reduction of pathogenic bacteria, as well as increased production of short-chain fatty acids (i.e., acetate, propionate and butyrate), and organic acids such as lactate, succinate, and pyruvate, that can be used by the pig as a source of energy (Zhou et al., 2021; Kiernan et al., 2023).

The efficiency of exogenous enzymes on nutrient digestibility is inconsistent and variable, depending on the enzymes used and the type of dietary fiber in the diets. Addition of xylanase or β-glucanase to diets for pigs may improve nutrient digestibility and growth performance (Torres-Pitarch et al., 2019; Petry et al., 2020; Galli et al., 2024); but in some cases, there appears to be no beneficial effect of enzyme supplementation in diets for pigs (Woyengo et al., 2008; Willamil et al., 2012; Casas and Stein, 2016). The combination of xylanase and β-glucanases may increase nutrient and energy digestibility in diets (Kiarie et al., 2012; Jerez-Bogota et al., 2020; Oliveira et al., 2022). Therefore, a novel xylanase and β-glucanase

combination was developed, but there are no data demonstrating the efficiency of this novel combination of enzymes on nutrient utilization by pigs fed diets high in fiber. Therefore, an experiment was conducted to test the hypothesis that supplementation of the novel combination of xylanase and β-glucanase in diets will increase the apparent total tract digestibility (**ATTD**) of energy and total dietary fiber (**TDF**), and therefore, also increase metabolizable energy (**ME**) when added to diets for growing pigs.

MATERIALS AND METHODS

The protocol for the experiment was submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois before the experiment was initiated. Pigs were the offspring of Line 800 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Experimental diets

A control diet containing wheat, barley, wheat middlings, soybean meal, and canola meal was formulated to meet nutrient requirements of growing pigs from 25 to 60 kg (NRC, 2012). Two additional diets were formulated by supplementing the control diet with the xylanase and β-glucanase combination in either a low dose or a high dose. Thus, a total of 3 diets were used (Tables 5.1, 5.2, and 5.3). The xylanase and β-glucanase combination (*LUNA XB101*) was supplied by Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands. The low dose of the enzymes combination provided 1,220 xylanase units (**XU**)/kg and 115 β-glucanase units (**BGU**)/kg, and the high dose of the enzymes provided 1,800 XU/kg and 170 BGU/kg to the diet. One xylanase unit is defined as the amount of enzyme that will release 0.48 μmole of xylose from arabinoxylan per min at pH 4.2 and 50 ºC. One β-glucanase unit is defined as the amount of

enzyme required to liberate 1 μmol of glucose per min at 40 °C in 100 mL buffer solution containing 1 g of β-glucan, pH 3.5. All dietary nutrients were included in the diets to meet or exceed current requirement estimates (NRC, 2012). All diets were fed in meal form. Throughout the experiment, pigs had free access to feed and water.

Animals, housing, and experimental design

Seventy-two growing barrows (initial body weight: 27.83 ± 2.16 kg) were allotted to 3 blocks with 24 pigs per block and allotted to one of the three diets with 8 replicate pigs per treatment in each block for a total of 24 pigs per treatment. In each block, pigs were housed individually in metabolism crates equipped with a self-feeder, a nipple waterer, slatted floors, and a screen floor, and a urine pan was installed under the slatted floor to allow for the total, but separate, collection of urine and feces. Pigs were fed at 3.2 times the energy requirement for maintenance (i.e., 197 kcal/kg × body weight$^{0.60}$; NRC, 2012), and daily feed allotments were provided each day in 2 equal meals at 0800 and 1600 h. Throughout the experiment, pigs had ad libitum access to water. Pigs were fed experimental diets for 15 days. The initial 6 days were considered the adaptation period to the diets, which was followed by 5 days of fecal collection using the marker-to-marker procedure (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., ferric oxide), which was included in the morning meal on day 7, appeared in the feces, and fecal collection ceased when the second marker (i.e., chromium oxide), which was included in the morning meal on day 12, appeared (Adeola, 2001). Urine was collected in buckets that were placed under the urine pans and 50 mL of 6*N* HCl were added to each bucket. Urine collection started at 0900 hours on day 7 and ceased at 0900 hours on day 12. Buckets were emptied daily, the weight of the collected urine was recorded, and 10% of the collected urine was stored at −20 °C until subsampling.

Pig weights were recorded at the beginning and at the conclusion of the experiment. Feed allowance and feed refusals were also recorded. Ten pigs were euthanized at the end of each period, 5 pigs fed the control diet, and 5 pigs fed the diet containing the high dose enzymes for a total of 15 pigs for each of the 2 treatments for the 3 blocks. A fresh ileal digesta sample was collected from euthanized pigs in falcon tubes of 50 mL immediately after euthanasia. This sample was placed on ice and immediately transferred to the laboratory for determination of digesta viscosity.

Chemical analyses

At the conclusion of the experiment, fecal samples were dried in a forced air oven (model Heratherm OMH750, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 55ºC and then ground through a 1-mm screen using a swing-type grain mill (model RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China). Ingredients and diets were also ground before analysis. Urine samples were thawed and mixed within animal, and a sub-sample was prepared for analysis following the procedure described by Kim et al. (2009). Approximately 10 mL of urine in quadruplicates was added to a cotton ball that was placed in a small plastic bag. The weights of the plastic bag, the cotton ball, and the plastic bag containing the cotton ball and urine were recorded, and the bag containing the cotton ball and urine was lyophilized (model Gamma 1-16 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and the weight was recorded again. Ingredients, diets, and fecal samples were analyzed for dry matter, which was determined by oven drying at 135ºC for 2 hours (method 930.15; AOAC Int., 2019), and for gross energy determined with bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA) using benzoic acid as the standard for calibration. To calculate the gross energy in the urine, the gross energy contributed by the plastic bag and the cotton ball were

subtracted from the total gross energy that was measured in the bag containing the cotton ball and the urine (Kim et al., 2009). Diets and ingredients were also analyzed for ash (method 942.05; AOAC Int., 2019), and acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3*N* HCl (AnkomHCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (method Am 5-04; AOCS, 2013) using petroleum ether (AnkomXT-15 Extractor, Ankom Technology, Macedon, NY, USA). Nitrogen was analyzed in diets and ingredients using the combustion procedure (method 990.03; AOAC Int, 2019) on a LECO FP628 Nitrogen Analyzer (Leco Corp., St. Joseph, MI, USA). Crude protein was calculated as 6.25 × nitrogen. Diets and ingredients were also analyzed for amino acids on an amino acid analyzer (model L8800 Hitachi Amino Acid Analyzer, Hitachi High Technologies America Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 hours at 110ºC (method 982.30 E(a); AOAC Int., 2019). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2019). Tryptophan was determined after NaOH hydrolysis for 22 hours at 110ºC (method 982.30 E(c); AOAC Int., 2019). Calcium, P, K, Mg, Na, Fe, Cu, Mn, and Zn in diets and ingredients were analyzed (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optimal emission spectrometry (ICP-OES, Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600ºC for 4 hours (method 942.05, AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. 49 Environmental Protection Agency, 1996). Starch was analyzed in diets and ingredients by the glucoamylase procedure (method 979.10; AOAC Int., 2019). Acid detergent fiber (method 973.18, AOAC Int., 2019) and neutral detergent fiber (method 2002.04, AOAC Int., 2019) were

determined in diets and ingredients using Ankom Technology methods 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY, USA). Diets, ingredients, and fecal samples were also analyzed for insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA) as established by method 991.43 (AOAC Int., 2019). Total dietary fiber was calculated as the sum of IDF and SDF. The particle size of diets was determined using 100 g of the diet sample placed on top of test sieves that were placed in a vibratory sieve shaker for 15 min. The weight of the material in each of the test sieves was recorded for the calculation of mean particle size (ANSI/ASAE, 2008). Diets were also analyzed for xylanase and β-glucanase activity using procedure F_019 and F_070 (Danisco Animal Nutrition, Brabrand, Denmark).

Viscosity was measured in ileal digesta samples using a procedure modified after Duarte et al. (2019) and was expressed as apparent viscosity in centipoise. In short, samples were collected into a 15 mL conical centrifuge tube and centrifuged at 1,000 × g at 4 °C for 10 min to obtain the liquid phase. The liquid phase was transferred to a 2 mL Eppendorf tube and centrifuged at 10,000 × g at 4 °C for 10 min and 0.5 mL of the supernatant was removed by suction. Viscosity of the supernatant was measured using a Brookfield LV-DV-2T viscometer (Brookfield Eng. Lab. Inc., Middleboro, MA, USA) with a Wells-Brookfield Cone/Plate extension and a CPA-40Z cone spindle. Values were reported as the average shear rate of 45/s. Viscosity was measured at room temperature (23 °C).

Calculations and statistical analyses

Following chemical analysis, ATTD of dry matter, gross energy, IDF, SDF and TDF was calculated for each diet, and the digestible energy (**DE**) and ME in each diet were calculated as well. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA)

with pig as the experimental unit. Homogeneity of the variances and normality of the residuals were confirmed using the UNIVARIATE procedure in PROC MIXED. Diet was the fixed effect, and block and pig replicate within block were random effects. Treatment means were calculated using the LSMEANS statement, and means were separated using the PDIFF statement with Tukey's adjustment (Tukey, 1977). Outliers were tested using the interquartile range method, as observations that deviated from the 1st or 3rd quartiles by more than 2.5 times the interquartile range (Tukey, 1977). A pig was excluded from statistical analysis if 3 or more response variables were identified as outliers. One pig fed the diet containing the high dose enzymes was identified as an outlier and removed but all other pigs were included in the final analysis. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

There were no differences in initial body weight, final body weight, or viscosity of ileal digesta of pigs fed experimental diets (Table 5.4). Dry matter intake, fecal dry matter output, analyzed dry matter in feces, and therefore, the ATTD of dry matter were also not different among diets (Table 5.5). Analyzed gross energy in feces was greater ($P < 0.05$) for pigs fed the control diet compared with pigs fed the enzyme supplemented diets and the ATTD of gross energy and DE tended to be greater ($P < 0.10$) in the diet containing the high dose of enzymes compared with the control diet. There were no differences in the urine output of gross energy. However, the diet containing the high dose enzymes had greater ($P < 0.05$) ME than the control diet. The intake of IDF, SDF, and TDF, as well as analyzed IDF, SDF, and TDF in feces, and IDF, SDF, and TDF fecal output were not different among treatments, and there were no differences in the ATTD of IDF, SDF, and TDF among treatments (Table 5.6).

DISCUSSION

Concentrations of dry matter, gross energy, ash, acid-hydrolyzed ether extract, starch, crude protein, amino acids, and minerals in ingredients were in agreement with reported values (NRC, 2012). Cereal grains including wheat and barley account for the majority of the energy in diets for pigs due to their high concentration of starch (50 to 75%; Stein et al, 2016). However, concentration and type of dietary fiber varies among ingredients. Total dietary fiber in wheat consist mainly of arabinoxylans (7% dry matter-basis) and cellulose (2% dry matter-basis), whereas TDF in barley contains arabinoxylans (8% dry matter-basis), mixed linked β-glucans (4% dry matter-basis) and cellulose (4% dry matter-basis; Navarro et al., 2019). Cereal coproducts have reduced starch concentration and increased dietary fiber concentration compared with the cereal grains (Bach Knudsen, 2014). Therefore, wheat middlings contain primarily arabinoxylans and cellulose, as wheat, but in greater concentrations (Jaworski et al., 2015). Oilseed meals, including soybean meal and canola meal, are used as protein sources due to their high concentrations of amino acids. However, the fiber in oilseed meals consist of cellulose, pectic polysaccharides, and galacto-oligosaccharides, whereas arabinoxylans are not present in oilseed meals (Lannuzel et al., 2022). Xylanase is expected to hydrolyze the xylose backbone in arabinoxylans, and because arabinoxylans is the major fiber component in wheat, barley, and wheat middlings, there was substrate in all diets for xylanase. Likewise, the mixed linked β-glucans in barley provide some substrate for the β-glucanase, although there was a lot less β-glucans in the diets compared with arabinoxylans. The analyzed nutrient composition and particle size of the diets used in the experiment were also in agreement with predicted values. The DE and ME determined for the control diet was close to calculated values from the DE and

ME in the ingredients used in the experiment (NRC, 2012). Xylanase and β-glucanase activities in the experimental diets were as expected.

Xylanase alone or in combination with β-glucanase added in wheat-based diets improved the digestibility of dry matter and energy in growing pigs (Kiarie et al., 2012, Dong et al., 2018; Abelilla and Stein, 2019; Torres-Pitarch et al., 2019; Zhang et al., 2020). The observation that ATTD of gross energy, and the DE and ME in diets supplemented with enzymes in high dose increased was a consequence of less gross energy analyzed in feces from pigs fed this diet. Both xylanase and β-glucanase may degrade the physical fiber matrix into smaller fiber fractions. The oligosaccharides released by the enzymes may be soluble when reaching the hindgut, allowing the microbes to use them as substrate for fermentation, and increasing the production of short-chain fatty acids that can be absorbed and utilized by the pig as energy source (Kiarie et al., 2012; Kiernan et al., 2023). Simultaneously, the hydrolysis of fiber by enzymes can increase access of endogenous digestive enzymes to nutrients that can be trapped in the fiber matrix, resulting in improved energy digestibility (de Lange et al., 2010; Abelilla and Stein, 2019).

It was hypothesized that carbohydrases may modify the solubility of fiber fractions, affecting the viscosity of the digesta of pigs (Lærke et al., 2015; Tiwari et al., 2019; Jang et al., 2024); however, no effects of dietary treatments on viscosity were observed, which is in agreement with some previous data (Passos et al., 2015; Duarte et al., 2021). It is possible that the soluble fiber fractions produced by the enzymes were not sufficient to result in a change in the viscosity of the digesta. Barley contains approximately 5% β-glucans (dry matter basis) but wheat contains only 1% (Bach Knudsen, 2014). Therefore, with 17.65% barley, and 25.20% wheat in the diets, the calculated concentration of β-glucans in the diets was less than 2%, which is likely the reason for the lack of a measurable effect of β-glucanase in the diets. Reductions in

viscosity typically are observed when pigs are fed diets with greater levels of β-glucans or arabinoxylans in soluble form (Duarte et al., 2019). However, the viscosity of digesta in pigs is considerably less than in poultry due to the greater concentration of water in pig digesta. Therefore, effects on viscosity of digesta in pigs due to exogenous enzymes may be negligible (Bedford and Schulze, 1998).

The lack of differences in the ATTD of dry matter and TDF in diets containing xylanase and β-glucanase may be a result of microbial growth in the hindgut of pigs, resulting in greater microbial mass excreted in the feces. Microbial mass can be analyzed as undigested dry matter, because it represents the portion of proteins, lipids, and carbohydrates that are present in microbial cells (Montoya et al., 2019). Likewise, inclusion of high-fiber ingredients in diets may influence the excretion of nondietary interfering materials (i.e., polysaccharides from yeast and bacteria; Montoya et al., 2016). These polysaccharides are also analyzed as TDF in the feces, resulting in interference in the calculation of digestibility of fiber (Cervantes Pahm et al., 2014; Montoya et al., 2016). As addition of enzymes in the diet for pigs may stimulate microbial growth in the hindgut, it may also result in greater excretion of nondietary interfering materials, resulting in no differences in ATTD of TDF. However, the enzyme activity results in better utilization of energy from the diet by pigs, due to better utilization of fibrous compounds for fermentation, which is in agreement with data indicating increase relative abundance of microbial communities that ferment oligosaccharides (Zhang et al., 2018; Petry et al., 2021).

CONCLUSIONS

Addition of a novel xylanase and β-glucanase combination in high-fiber diets for growing pigs tended to increase ATTD of gross energy and DE and increased ME in diets with the high

enzyme dose, indicating that the enzymes result in increased energy utilization by pigs fed diets containing high-fiber ingredients. However, the lack of effects of enzymes on fiber digestibility is likely a result of increased fecal excretion of microbial mass from pigs fed diets containing enzymes. Further research is needed to understand the effects of the combination of xylanase and β-glucanase supplementation on the digestibility of TDF and the utilization of oligosaccharides produced by enzymatic hydrolysis of fiber.

TABLES

Table 5.1. Analyzed composition of main ingredients in diets, as-fed basis

Item	Wheat	Barley	Soybean meal	Canola meal	Wheat middlings
Gross energy, kcal/kg	3,930	4,014	4,374	4,313	4,162
Dry matter, %	89.62	88.88	90.83	89.17	89.16
Ash, %	0.83	2.01	6.17	6.17	3.64
Acid hydrolyzed ether extract, %	1.70	2.92	3.89	5.52	5.59
Crude protein, %	9.71	12.96	46.03	36.65	16.72
Starch, %	65.51	47.23	0.86	0.33	25.78
Neutral detergent fiber, %	5.10	13.01	7.04	22.26	28.08
Acid detergent fiber, %	2.36	4.78	4.10	17.15	9.38
Total dietary fiber, %	8.30	23.10	15.30	27.30	33.20
Insoluble dietary fiber, %	7.60	15.20	14.00	26.30	31.70
Soluble dietary fiber, %	0.70	7.90	1.30	1.00	1.50
Minerals					
Ca, %	0.02	0.05	0.32	0.60	0.09
P, %	0.14	0.39	0.73	1.15	0.77
K, %	0.15	0.45	2.02	1.01	0.74
Mg, %	0.03	0.13	0.28	0.55	0.30
Na, %	0.01	0.03	0.02	0.04	0.01
Fe, mg/kg	21.17	79.03	128.51	134.32	135.74
Zn, mg/kg	6.82	16.37	53.33	39.55	30.23

Table 5.1. (cont.)

Cu, mg/kg	16.15	38.80	59.83	67.93	84.63
Mn, mg/kg	17.72	32.44	46.76	75.10	117.08
Indispensable amino acids, %					
Arg	0.45	0.67	3.55	2.44	1.44
His	0.25	0.32	1.40	1.16	0.48
Ile	0.37	0.49	2.44	1.71	0.63
Leu	0.66	0.89	3.88	2.86	1.13
Lys	0.34	0.53	3.18	2.32	0.78
Met	0.17	0.23	0.68	0.80	0.29
Phe	0.44	0.70	2.62	1.68	0.75
Thr	0.28	0.43	1.90	1.68	0.57
Trp	0.11	0.12	0.62	0.44	0.16
Val	0.43	0.66	2.50	2.15	0.85
Dispensable amino acids, %					
Ala	0.35	0.52	2.13	1.73	0.84
Asp	0.47	0.79	5.55	2.83	1.21
Cys	0.25	0.29	0.70	1.05	0.38
Glu	2.90	3.13	9.11	7.20	3.87
Gly	0.39	0.53	2.07	2.05	0.90
Pro	0.99	1.39	2.54	2.53	1.30
Ser	0.42	0.47	2.01	1.37	0.65
Tyr	0.27	0.35	1.87	1.15	0.46

Table 5.2. Ingredient composition of experimental diets, as-fed basis

Ingredient, %	Control	Low enzymes dose[1]	High enzymes dose[1]
Wheat	25.30	25.20	25.20
Barley	17.65	17.65	17.65
Soybean meal	16.92	16.92	16.92
Canola meal	7.00	7.00	7.00
Wheat middlings	30.00	30.00	30.00
Soybean oil	0.50	0.50	0.50
Limestone	1.10	1.10	1.10
Monocalcium phosphate	0.81	0.81	0.81
L-Lys HCl	0.18	0.18	0.18
L-Thr	0.04	0.04	0.04
Salt	0.30	0.30	0.30
Low dose enzymes premix[2]	-	0.10	-
High dose enzymes premix[3]	-	-	0.10
Mineral premix[4]	0.10	0.10	0.10
Vitamin premix[5]	0.10	0.10	0.10

[1]Enzymes = xylanase and β-glucanase combination (LUNA XB101); Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands.

[2]The low dose enzymes premix contained 0.98 million xylanase units (XU)/kg and 98,000 β-glucanase units (BGU)/kg (0.043 kg of enzymes concentrate containing 22.5 million XU/kg and 2.25 million BGU/kg was mixed with 0.037 kg of wheat bran and 0.72 kg of soybean meal). At 0.1% inclusion, the low enzymes dose diet was expected to contain approximately 1,220 XU/kg

Table 5.2. (cont.)

and 120 BGU/kg. One XU is defined as the amount of enzyme that will release 0.48 μmole of xylose from arabinoxylan per min at pH 4.2 and 50 ºC. One BGU is defined as the amount of enzyme required to liberate 1 μmol of glucose per min at 40 °C in 100 mL buffer solution containing 1 g of β-glucan, pH 3.5.

[3]The high dose enzymes premix contained 1.44 million XU/kg and 144,000 BGU/kg (0.064 kg of enzymes concentrate containing 22.5 million XU/kg and 2.25 million BGU/kg was mixed with 0.016 kg of wheat bran and 0.72 kg of soybean meal). At 0.1% inclusion, the high enzymes dose diet was expected to contain 1,800 XU/kg and 180 BGU/kg.

[4]The micromineral premix provided the following microminerals per kg complete diet: manganese, 50 mg from $MnSO_4 \cdot H_2O$; iron, 50 mg from $FeSO_4 \cdot H_2O$; zinc, 50 mg from ZnO; copper, 3.33 mg from $CuSO_4 \cdot 5H_2O$; iodine, 0.50 mg from ethylene diamine dihydroiodide; selenium, 0.07 mg from Na_2SeO_3.

[5]The vitamin premix provided the following quantities of vitamins and per kg of complete diet: Vitamin A as retinyl acetate, 3,300 IU; vitamin D_3 as cholecalciferol, 330 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 44 IU; vitamin K as menadione nicotinamide bisulfate, 2.2 mg; riboflavin, 4.4 mg; vitamin B_{12}, 0.02 mg; $_D$-pantothenic acid as $_D$-calcium pantothenate, 12.1 mg; niacin, 16.5 mg; choline as choline chloride, 142.89 mg.

Table 5.3. Analyzed composition of experimental diets, as-fed basis

Item	Control	Low enzymes dose[1]	High enzymes dose[1]
Gross energy, kcal/kg	4,063	4,062	4,072
Dry matter, %	89.97	89.53	89.62
Ash, %	4.97	5.02	5.01
Acid hydrolyzed ether extract, %	4.22	4.15	4.21
Crude protein, %	19.97	19.89	19.94
Starch, %	32.34	31.97	30.78
Neutral detergent fiber, %	17.86	16.61	16.10
Acid detergent fiber, %	7.11	6.52	6.89
Total dietary fiber, %	25.40	23.30	26.55
Insoluble dietary fiber, %	22.55	20.65	24.90
Soluble dietary fiber, %	2.85	2.65	1.65
Minerals			
Ca, %	0.72	0.74	0.72
P, %	0.86	0.86	0.88
K, %	0.91	0.90	0.90
Mg, %	0.27	0.27	0.27
Na, %	0.12	0.12	0.12
Fe, mg/kg	231.95	225.88	220.63
Zn, mg/kg	134.30	132.56	131.47
Cu, mg/kg	29.64	33.67	31.59

Table 5.3. (cont.)

Mn, mg/kg	102.55	118.81	112.89
Indispensable amino acids, %			
Arg	1.31	1.34	1.31
His	0.53	0.54	0.53
Ile	0.82	0.82	0.80
Leu	1.48	1.48	1.45
Lys	1.22	1.22	1.19
Met	0.32	0.31	0.30
Phe	0.95	0.96	0.94
Thr	0.77	0.79	0.77
Trp	0.21	0.21	0.21
Val	1.01	1.00	0.98
Dispensable amino acids, %			
Ala	0.93	0.93	0.91
Asp	1.75	1.77	1.74
Cys	0.40	0.41	0.40
Glu	4.10	4.16	4.10
Gly	0.95	0.97	0.95
Pro	1.35	1.35	1.34
Ser	0.84	0.86	0.84
Tyr	0.60	0.60	0.59
Particle size, μm	488	467	466

Table 5.3. (cont.)

Enzymes activity			
Xylanase, XU[2]/kg	< 160	992	1,965
β-glucanase, BGU[2]/kg	< 81	107	208

[1]Enzymes = xylanase and β-glucanase combination (LUNA XB101); Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands.

[2]One XU is defined as the amount of enzyme that will release 0.48 μmole of xylose from arabinoxylan per min at pH 4.2 and 50 ºC. One BGU is defined as the amount of enzyme required to liberate 1 μmol of glucose per min at 40 °C in 100 mL buffer solution containing 1 g of β-glucan, pH 3.5.

Table 5.4. Body weight, and viscosity of ileal digesta from pigs fed experimental diets[1]

Item	Control	Low enzymes dose[2]	High enzymes dose[2]	SEM	*P*-value
Initial body weight, kg	27.74	27.87	27.85	0.46	0.578
Final body weight, kg	38.45	38.86	39.09	0.56	0.349
Ileal digesta viscosity, centipoise	3.98	-	3.63	0.33	0.452

[1]Data are least squares means of 24 observations per treatment, except for ileal digesta viscosity when there were 15 observations for the control and the high enzymes dose treatments.

[2]Enzymes = xylanase and β-glucanase combination (LUNA XB101); Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands.

Table 5.5. Apparent total tract digestibility (ATTD) of dry matter and gross energy, and concentrations of digestible energy (DE) and metabolizable energy (ME) in experimental diets fed to pigs[1]

Item	Control	Low enzymes dose[2]	High enzymes dose[2]	SEM	*P*-value
Dry matter intake, kg/d	1.27	1.27	1.29	0.02	0.551
Fecal dry matter output, kg/d	0.24	0.24	0.23	0.01	0.346
Dry matter in feces, %	94.40	94.39	94.50	0.42	0.919
ATTD of dry matter, %	80.95	81.06	81.79	0.66	0.126
Gross energy intake, kcal/d	5,773	5,777	5,829	70	0.551
Gross energy fecal output, kcal/d	1,159	1,138	1,098	36	0.168
Gross energy in feces, kcal/kg	4,510^{a}	4,442^{b}	4,431^{b}	19	0.007
ATTD of gross energy, %	79.90^{y}	80.32^{xy}	81.14^{x}	0.77	0.058
Urine output, kg/d	5.28^{a}	3.24^{b}	4.90^{a}	1.01	0.017
Gross energy urine output, kcal/d	201	176	194	15	0.229
Gross energy in urine, kcal/kg	52	71	60	16	0.197
DE, kcal/kg	3,249^{y}	3,266^{xy}	3,299^{x}	31	0.058
ME, kcal/kg	3,105^{b}	3,142^{ab}	3,164^{a}	31	0.040

$^{x\text{-}y}$Values within a row lacking a common superscript tend to be different ($P < 0.10$).

$^{a\text{-}b}$Values within a row lacking a common superscript letter are different ($P < 0.05$).

[1]Data are least squares means of 24 observations per treatment, except for the high enzymes dose treatment (n = 23).

Table 5.5. (cont.)

[2]Enzymes = xylanase and β-glucanase combination (LUNA XB101); Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands.

Table 5.6. Apparent total tract digestibility (ATTD) of insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and total dietary fiber (TDF) in experimental diets fed to pigs[1]

Item	Control	Low enzymes dose[2]	High enzymes dose[2]	SEM	*P*-value
IDF intake, kg/d	0.32	0.32	0.33	0.01	0.551
IDF fecal output, kg/d	0.12	0.12	0.12	0.01	0.703
Fecal IDF, %	45.80	45.15	46.55	0.64	0.136
ATTD of IDF, %	63.56	64.30	64.66	1.16	0.417
SDF intake, kg/d	0.03	0.03	0.03	0.01	0.551
SDF fecal output, kg/d	0.01	0.01	0.01	0.01	0.226
Fecal SDF, %	3.20	3.80	3.59	0.56	0.205
ATTD of SDF, %	75.95	71.65	74.60	4.32	0.229
TDF intake, kg/d	0.36	0.36	0.36	0.01	0.551
TDF fecal output, kg/d	0.13	0.13	0.12	0.01	0.849
Fecal TDF, %	49.00	48.95	50.13	1.05	0.264
ATTD of TDF, %	64.77	65.02	65.63	1.34	0.583

[1]Data are least squares means of 24 observations per treatment, except for the high enzymes dose treatment (n = 23).

[2]Enzymes = xylanase and β-glucanase combination (LUNA XB101); Danisco Animal Nutrition and Health, Oegstgeest, The Netherlands.

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CHAPTER 6: GROWTH PERFORMANCE AND TOTAL TRACT DIGESTIBILITY OF NUTRIENTS BY WEANLING PIGS ARE IMPROVED BY AN EXOGENOUS XYLANASE AND A STIMBIOTIC REGARDLESS OF SOWS CONSUMPTION OF XYLANASE DURING LACTATION

ABSTRACT

Exogenous xylanase can increase utilization of fiber and energy when included in diets for pigs, and xylo-oligosaccharides (**XOS**) may improve growth performance of pigs by modulating intestinal fermentation. However, it is unclear if a stimbiotic (i.e., a combination of xylanase and **XOS**) has superior effects compared with a xylanase alone, and there is a lack of data demonstrating if xylanase fed to lactating sows influences growth performance of weanling pigs. Therefore, two hypotheses were tested: 1) xylanase and stimbiotic improve growth performance, apparent total tract digestibility (**ATTD**) of gross energy (**GE**) and total dietary fiber (**TDF**), digestible energy (**DE**), and intestinal health of weanling pigs and 2) offspring of sows fed xylanase in lactation have greater growth performance after weaning than offspring of sows fed no xylanase during lactation. One hundred and twenty pigs were weaned from sows fed a diet without xylanase, and 120 pigs were weaned from sows fed a lactation diet containing 16,000 beechwood xylanase units per kg (initial weight: 5.81 ± 0.50 kg). Pigs were allotted to a 2 × 3 factorial with two sow groups (lactation diet without or with xylanase) and three dietary treatments (i.e., control, control plus xylanase, or control plus stimbiotic). There were no interactions between sow treatment and post-weaning pig treatment, and sow treatment did not impact post-weaning growth or ATTD of GE and TDF in weaned pigs. From d 15 to 28 post-weaning, the ADG, G:F, ATTD of GE and TDF, and concentration of DE were greater ($P <$

0.05) for pigs fed the diet with stimbiotic than if fed the xylanase diet or the control diet, and pigs fed the xylanase diet had greater ($P < 0.05$) ADG, G:F, ATTD of GE and TDF, and concentration of DE than pigs fed the control diet. From d 29 to 42 post-weaning, pigs fed the diets with xylanase or stimbiotic had greater ($P < 0.05$) ADG, ATTD of GE and TDF, and DE than pigs fed the control diet. Pigs fed xylanase or stimbiotic had greater ATTD of gross energy and TDF, greater DE, and greater overall ADG, G:F, and final body weight on d 42 post-weaning than pigs fed the control diet, but feeding sows xylanase in lactation did not influence post-weaning growth performance.

Keywords: digestibility, growth performance, sows, stimbiotic, weanling pigs, xylanase.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BXU, beechwood xylanase units; cDNA, complementary deoxyribonucleic acid; DE, digestible energy; EDTA, ethylenediaminetetraacetic acid; G:F, gain to feed ratio; GE, gross energy; GIP, gastric inhibitory polypeptide; IDF, insoluble dietary fiber; IFN-γ, interferon-gamma; IL, interleukin; PYY, peptide YY; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RNA, ribonucleic acid; SDF, soluble dietary fiber; TDF, total dietary fiber; TNFα, tumor necrosis factor alpha; VH:CD, villus height to crypt depth ratio; XOS, xylo-oligosaccharides.

INTRODUCTION

Arabinoxylans, a polysaccharide made up of a chain of xylose units with sidechains of arabinose, galactose, and acetyl group, make up the majority of the fiber in cereal grains and cereal co-products (Jaworski et al., 2015). Arabinoxylans cannot be hydrolyzed by endogenous

enzymes, thus they pass through the small intestine undigested, but interfere with nutrient digestibility, water absorption, and digesta passage time (Baker et al., 2021). Exogenous xylanase hydrolyzes the β-(1− 4) glycosidic bonds between xylose units in the backbone of arabinoxylan, which results in the production in situ of arabinoxylo-oligosaccharides (**AXOS**). Xylo-oligosaccharides (**XOS**) and AXOS and may be fermented by intestinal microbes in the hindgut to release short-chain fatty acids that can provide energy to the animals (Navarro et al., 2019). Because of the stimulation of fermentation that is initiated by xylanase, utilization of dietary fiber and energy may be increased if xylanase is included in the diet, resulting in greater growth performance and improved gut barrier integrity of weanling pigs (Tiwari et al., 2018; Duarte et al., 2019; Chen et al., 2020, He et al., 2020). Xylo-oligosaccharides and AXOS are carbohydrates made up of 2 to 10 xylose units linked through β-(1→4)-linkages and sidechains of arabinose units or acetyl groups (Samanta et al., 2015; Chen et al., 2021b). Xylo-oligosaccharides may improve growth performance of weanling pigs because they can modulate the gut microbiota at very low doses (Pan et al., 2019; Chen et al., 2021a; Pang et al., 2021; González-Solé et al., 2022). A stimbiotic (i.e., the combination of xylanase and XOS) may also improve growth performance of weanling pigs by shifting the intestinal microbiome to favor fermentation of fiber (Cho et al., 2020), but it is not known if the effect of a stimbiotic is greater than that of xylanase.

Feeding xylanase to lactating sows may result in increased energy and nutrient digestibility (Acosta et al., 2024; Zhou et al., 2018), and possibly also a change in intestinal microbiome, which may influence the microbiome of nursing pigs. It is, therefore, possible that feeding xylanase to lactating sows results in a carry-over effect on growth performance, digestibility of nutrients, and intestinal health of weanling pigs, but this hypothesis has not been

investigated. Therefore, an experiment was conducted to test the hypothesis that xylanase alone or a stimbiotic improves growth performance and the apparent total tract digestibility (**ATTD**) of dry matter, gross energy (**GE**), crude protein, and total dietary fiber (**TDF**), and the concentration of digestible energy (**DE**) of diets, and intestinal health of weanling pigs. The second hypothesis was that the effect of using xylanase in post-weaning diets is greater in offspring of sows fed xylanase in lactation than in offspring of sows that were not fed xylanase during lactation.

MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was reviewed and approved prior to initiation of the experiment. Pigs used in this experiment were the offspring of Camborough sows mated to Line 800 boars (Pig Improvement Company, Hendersonville, TN, USA).

Animals, housing, experimental design and diets

One hundred twenty pigs were weaned from sows fed a control diet without xylanase and 120 pigs were weaned from sows fed a diet containing 16,000 beechwood xylanase units (**BXU**) per kg of an exogenous xylanase (240 weaned pigs in total; initial body weight: 5.81 kg ± 0.50 kg). One beechwood xylanase unit is defined as the amount of enzyme that will release 0.06 micromoles of reducing sugars (xylose equivalents) from beechwood xylan per min at pH 5.3 and 50 ºC. Sows had been fed experimental diets for two reproductive cycles and pigs used in this experiment were weaned at the end of the second cycle. Details of sow treatments and performance were published elsewhere (Acosta et al., 2024). Pigs were weaned in 4 blocks of 60 pigs per block, and weaning group was used as the blocking factor. Pigs in each block were

placed in a separate weaning units; thus there were a total of 4 weaning barns used in the experiment. The temperature in the barns was 30 ºC in week one after weaning, 28 ºC in week 2 after weaning, the temperature was then reduced by 1 ºC in each of the following weeks post-weaning. Pigs were housed in mixed-sex pens in groups of 5 pigs per pen, and sex was balanced among treatments. There were 6 treatments and 8 replicate pens per treatment. Pens had fully slatted floors, a feeder, and a nipple drinker. The experimental design was a 2 × 3 factorial with two sow treatments (sows fed the diet without xylanase and sows fed the diet with xylanase) and three dietary treatments after weaning (i.e., control, control plus 100 g/t of xylanase, or control plus 100 g/t of stimbiotic). Xylanase (Econase XT 25; AB Vista, Marlborough, UK) and stimbiotic (Signis; AB Vista, Marlborough, UK) were included in diets for weanling pigs as recommended by the supplier. The 100 g/t of xylanase in the diets was expected to provided 16,000 BXU of xylanase in the xylanase and the stimbiotic treatments. A 3-phase feeding program was used. Days 1 to 14 were phase 1, d 15 to 28 were phase 2, and d 29 to 42 were phase 3. Diets were based on corn, soybean meal, and wheat middlings, and phase 1 and phase 2 diets also contained fermented soybean meal (Industrial De Oleaginosas Americanas S.A., Barranca, Costa Rica) and lactose, and phase 1 diets contained fish meal as well (Table 6.1). Pigs were fed experimental diets in all three phases (Tables 6.2 and 6.3). All diets were formulated to meet nutrient requirements for weanling pigs (NRC, 2012), and all diets were fed as mash. Titanium dioxide (0.4 %) was included in all diets as an indigestible marker. Pigs were allowed *ad libitum* intake of feed and water throughout the experiment.

Collection of samples and data

Individual pig weights were recorded on the d of weaning, on d 14, d 28, and d 42. Feed addition to each pen was recorded daily, and the weight of feed left in the feeder was recorded on

d 14, d 28, and d 42. Diarrhea scores were assessed visually per pen every other d using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Diarrhea frequency was obtained by totaling the number of pen d with diarrhea scores ≥ 3 divided by the total number of pen d multiplied by 100.

On d 14, the pig in each pen with a body weight closest to the pen average was identified (4 barrows and 4 gilts per treatment) and sacrificed via captive bolt stunning. Ileal tissue samples between 2 and 3 cm long were collected approximately 80 cm from the ileal-cecal junction. Samples were cut and pinned with the serosa side down on a piece of cardboard and then fixed in 10% neutral buffered formalin for morphological evaluation and immunohistochemistry staining. Ileal mucosa samples were washed with phosphate-buffered saline, scraped gently, snap-frozen in liquid N, and stored at -80 ºC until used for ribonucleic acid (**RNA**) extraction.

On d 28, two blood samples were collected via vena puncture from 1 pig per pen. One sample was collected in a vacutainer with heparin, and the other sample was collected in a vacutainer with ethylenediaminetetraacetic acid (**EDTA**). Both samples were centrifuged at 2,000 × g at 4 ºC for 15 min to yield blood plasma, which was stored at -20 ºC until analyzed. Fecal samples were collected from all pigs for 3 d (d 26 to 28) in phase 2, and for 3 d (d 40 to 42) in phase 3 via anal stimulation. Collected fecal samples were stored at -20 ºC until processed.

Chemical Analyses

At the conclusion of the experiment, fecal samples from each pen were thawed and dried in a 50˚C forced-air drying oven, and ground using a grain mill (model: RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China) before analysis. Samples of the major ingredients used in the diets and a sample of all diets were collected at the time of diet mixing and ground before analysis. Ingredients, diets, and fecal samples were analyzed for dry matter

determined by oven drying at 135 ºC for 2 h (method 930.15; AOAC Int., 2019), and diets and ingredients were also analyzed for ash (method 942.05; AOAC Int., 2019). Acid hydrolyzed ether extract was analyzed in diets and ingredients by acid hydrolysis using 3*N* HCl (AnkomHCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (method 2003.06; AOAC Int., 2019) using petroleum ether (AnkomXT-15 Extractor, Ankom Technology, Macedon, NY, USA). Diets and ingredients were also analyzed for amino acids on an amino acid analyzer (model L8800 Hitachi Amino Acid Analyzer, Hitachi High Technologies America Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110 ºC (method 982.30 E(a); AOAC Int., 2019). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2019). Tryptophan was determined after NaOH hydrolysis for 22 h at 110 ºC (method 982.30 E(c); AOAC Int., 2019). Calcium, P, K, Mg, Na, Cu, Fe, Mn, and Zn in diets and ingredients were analyzed (method 985.01 A, B, and C; AOAC Int., 2019) using inductively coupled plasma-optimal emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 ºC for 4 h (method 942.05, AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 1996). Ingredients, diets and fecal samples were analyzed for GE determined with bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA) using benzoic acid as the standard for calibration. Ingredients, diets, and fecal samples were analyzed for nitrogen measured using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 Nitrogen Analyzer (Leco Corp., St. Joseph, MI, USA). Crude protein was calculated as 6.25 × nitrogen. Starch was analyzed in diets, ingredients, and fecal samples by the glucoamylase procedure

(method 979.10; AOAC Int., 2019). These samples were also analyzed for insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) according to method 991.43 AOAC Int., 2019 using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of IDF and SDF. Fecal samples and diets were analyzed for titanium (Fowler et al., 2022). Diets were analyzed for xylanase activity using the QuantiPlate ELISA kit specific for Econase XT (ESC Standard Analytical Method SAM115; AB Vista, Plantation, FL, USA).

Heparinized plasma samples were analyzed for plasma urea N, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Plasma EDTA samples were analyzed for peptide YY (**PYY**) and gastric inhibitory polypeptide (**GIP**) using assay kits according to manufacturer specifications (Phoenix Pharmaceuticals Inc.; My BioSource, San Diego, CA, USA; respectively). The EDTA samples were also analyzed for tumor necrosis factor-alpha (**TNFα**), interferon-gamma (**IFNγ**), and interleukin (**IL**) 1α, 1β, 1RA, 2, 4, 6, 8, 10, 12, and 18 using a cytokine/chemokine magnetic bead panel according to manufacturer specifications (MILLIPLEX Porcine Cytokine/Chemokine Magnetic Bead Panel; EMD Millipore, Darmstadt, Germany).

Morphology was measured in ileal tissue (Espinosa et al., 2021). After fixation, each ileal tissue sample was cut into 2 to 3 mm thick cross-sections and embedded in paraffin for slide preparation. From each sample, three to four transverse sections were selected and stained with hematoxylin and eosin. Slides were scanned using a 2.0-HT NanoZoomer (Hamamatsu, Bridgewater, NJ, USA) and 10 intact, well-oriented villi and associated crypts were identified. Villus height, crypt depth, and lamina propria thickness of the ileum were measured of each

sample in duplicate using an image processing and analysis system (NDP.View2, Hamamatsu, Bridgewater, NJ, USA). Villus height:crypt depth (**VH:CD**) was also calculated.

The RNA was extracted from 30 ± 0.2 mg of frozen ileal mucosa using β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) according to the RNeasy Mini Kit (QIAGEN, Germantown, MD, USA) manufacturer's instructions (Espinosa et al., 2021). Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The RNA quality was determined using a Fragment AnalyzerTM Automated CE System (Method DNF-471-33-SS Total RNA 15nt; Advanced Analytical, Ankeny, IA, USA), and RNA samples with an RNA quality number greater than 7 were diluted to 100 ng/μL with nuclease-free water and used for complementary deoxyribonucleic acid (**cDNA**) synthesis. The cDNA was then diluted 1:4 with nuclease-free water to conduct quantitative reverse-transcription polymerase chain reaction (**qRT-PCR**) analysis which was performed using 4 μL of diluted cDNA and 6 μL of a mixture including forward and reverse primers, SYBR Green master mix (Quanta Biosciences Inc., Gaithersburg, MD, USA) and nuclease-free water in a MicroAmpTM Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA, USA). Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (Gonzalez et al., 2013), and hypoxanthine-guanine phosphoribosyl transferase (Nygard et al., 2007), were used to normalize the abundance of tested genes (Table 6.4). Tested genes included occludin and zonula occludens-1 because these genes regulate intestinal permeability and paracellular absorption of nutrients (Hu et al., 2013).

Calculations and statistical analyses

At the end of the experiment, data were summarized to calculate average daily feed intake (**ADFI**), average daily gain (**ADG**), and gain:feed ratio (**G:F**) within each pen and

treatment group. Data were summarized for d 1 to 14, d 15 to 28, d 29 to 42, and for the entire experiment. If a pig was removed from a pen during the experiment, ADFI and G:F were adjusted for the feed consumed by the pig that was removed, as described by Lindemann and Kim (2007).

Apparent total tract digestibility of dry matter, GE, starch, crude protein, IDF, SDF, and TDF were calculated for each diet for phases 2 and 3, and the concentration of DE in each diet and phase was also calculated (Adeola, 2001). Data from the qRT-PCR analysis were analyzed using QuantStudio™ Real-time PCR software (version 1.3; Applied Biosystems, Foster City, CA), using the relative standard curve method for quantification (Livak and Schmittgen, 2001).

Data were analyzed in a 2 × 3 factorial using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Pen was the experimental unit for growth performance and digestibility of nutrients and energy, whereas the pig was the experimental unit for all markers of intestinal health. The model included sow treatment, post-weaning dietary treatment, and the interaction between sow group and post-weaning dietary treatment as fixed effects, and block and replicate within block as random effects. However, for all response parameters, no interactions were observed, therefore, the final model only included sow treatment and post-weaning dietary treatment as main effects. Normality of the residuals was confirmed, and outliers were identified using the UNIVARIATE procedure of SAS. Outliers were defined as observations with internally studentized residuals less than 3 or greater than 3, but no were identified. Least square means were calculated for each independent variable, and means were separated using the PDIFF option with Tukey adjustment (Tukey, 1997). The Chi-squared test was used to analyze the frequency of diarrhea among treatments. Least squares means were calculated and means were

separated for the frequency of diarrhea data using GENMOD procedure of SAS. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

Growth performance

There were no effects of sow diet treatment on growth performance parameters of pigs during the 42- d post-weaning period (Table 6.5). Phase 1 ADG, ADFI, G:F, and body weight were not affected by post-weaning dietary treatments. Phase 2 ADG and G:F were greater ($P < 0.05$) for pigs fed diets with stimbiotic compared with pigs fed the control diet or the diet with xylanase, but pigs fed the diet with xylanase had greater ($P < 0.05$) G:F than pigs fed the control diet. At the conclusion of phase 2, body weight tended to be greater ($P < 0.10$) for pigs fed the diet with stimbiotic compared with pigs fed the control diet. During phase 3, pigs fed diets with stimbiotic or xylanase had greater ($P < 0.05$) ADG compared with pigs fed the control diet, and ADFI tended to be greater ($P < 0.10$) by pigs fed the diet with stimbiotic than by pigs fed the control diet. Pigs fed a diet with stimbiotic or xylanase also tended to have greater ($P < 0.10$) G:F compared with pigs fed the control diet. For the entire experimental period, pigs fed diets with stimbiotic or xylanase had greater ($P < 0.05$) ADG, G:F, and final body weight on d 42 post-weaning than pigs fed the control diet.

There were no differences among treatments in diarrhea scores in phases 1, 2, or overall, but during phase 3, pigs from sows fed a diet without xylanase in lactation tended to have reduced ($P < 0.10$) diarrhea scores compared with pigs from sows fed a diet with xylanase in lactation (Table 6.6). During phase 3, pigs fed the control diet also had lower ($P < 0.05$) diarrhea scores compared with pigs fed a diet containing stimbiotic or xylanase. There were no

differences in the frequency of diarrhea by pigs from sows fed a diet without or with xylanase in phases 1, 2, or overall; however, the frequency of diarrhea during phase 1 tended to be reduced ($P < 0.10$) in pigs fed the diet with xylanase compared with pigs fed the other diets, but in phase 2, pigs fed the control diets tended to have lower ($P < 0.10$) frequency of diarrhea compared with pigs fed the other diets. During phase 3, the frequency of diarrhea was reduced ($P < 0.05$) in pigs fed the control diet compared with pigs fed the diets containing xylanase or stimbiotic.

Digestibility of nutrients

No effects of sow treatment on digestibility of energy or nutrients during the 42- d post-weaning period were observed (Table 6.7). The ATTD of dry matter, GE, crude protein, IDF, and TDF in phase 2 was greater ($P < 0.05$) by pigs fed the diet with stimbiotic compared with pigs fed the diet with xylanase or the control diet. Likewise, the concentration of DE was greater ($P < 0.05$) in the diet with stimbiotic than in the other two diets. In phase 3, pigs fed diets with stimbiotic or xylanase had greater ($P < 0.05$) ATTD of dry matter, GE, IDF, and TDF, and greater ($P < 0.05$) DE than pigs fed the control diet. The ATTD of crude protein was also greater ($P < 0.05$) in the diet with stimbiotic than in the control diet. The ATTD of starch and SDF did not differ among treatments in phases 2 and 3.

Markers of intestinal health

In phase 2, pigs fed the diet with xylanase tended to have reduced ($P < 0.10$) plasma urea N compared with pigs fed the diet with stimbiotic (Table 6.8). Plasma total protein concentration was reduced ($P < 0.05$) in pigs from sows fed the diet with xylanase in lactation compared with pigs from sows fed a diet without xylanase, but pigs fed the diet with stimbiotic had greater ($P < 0.05$) plasma total protein and albumin than pigs fed the control diet. Pigs fed the diet with xylanase had reduced ($P < 0.05$) IL-1α, IL-2, IL-4, and IL-10 in plasma, and tended to have

reduced ($P < 0.10$) IL-1β compared with pigs fed the other diets. However, pigs from sows fed xylanase in lactation had reduced ($P < 0.05$) IL-1RA and tended to have reduced ($P < 0.10$) IL-8 compared with pigs from sows fed a diet without xylanase. No differences were observed in villus height, crypt depth, lamina propria thickness, VH:CD, or mRNA abundance of occludin and zonula occludens-1 among treatment groups at the end of phase 1 (Tables 6.9 and 6.10).

DISCUSSION

Ingredients and diets composition

Concentrations of dry matter, crude protein, amino acids, GE, starch, IDF, SDF, ash, and acid-hydrolyzed ether extract of ingredients were in agreement with reported values (NRC, 2012). Most fiber in corn and wheat middlings consists of arabinoxylans (Jaworski et al., 2015), which is poorly fermented by pigs; however, exogenous xylanase hydrolyzes the β-1,4-glycosidic bonds in arabinoxylans, releasing oligosaccharides and pentoses that potentially can be fermented by hindgut microbes with subsequent synthesis of volatile fatty acids that can be used by pigs (Petry and Patience, 2020). Therefore, the ingredients used in this experiment provided the substrate for the xylanase enzyme supplemented alone or in combination with XOS. The xylanase activity for the control diets did not exceed the detection limit (2,000 BXU/kg), and the average xylanase activity for the diets with xylanase (14,200 ± 700 BXU/kg) and stimbiotic (19,370 ± 3,800 BXU/kg) was in agreement with the expected values. Likewise, the analyzed xylanase activity of the sow lactation diets was in agreement with calculated values (data not shown; Acosta et al., 2024).

Growth performance and nutrient digestibility

Diets rich in dietary fiber generally have lower nutritive value and sometimes this results in reduced growth performance of weanling pigs (Agyekum and Nyachoti, 2017). Incorporating exogenous enzymes and prebiotics in the diet of pigs may increase nutrient digestion and absorption, resulting in enhanced growth performance (Duarte et al., 2019, Chen et al., 2021a; 2021b).

The lack of effects of xylanase and stimbiotic on growth performance during phase 1 of this experiment was in agreement with data from experiments using grading levels of XOS (Pan et al., 2019; Chen et al., 2021a), but in disagreement with data reporting greater ADG when XOS were added to the diets from d 1 to 14 after weaning (Liu et al., 2018). This discrepancy may have been due to differences in the xylanase and stimbiotic that were used, the doses or activity of the products, or the composition of the diets. It is also possible that animals needed to adapt to the enzyme and the stimbiotic (Petry et al., 2024), or needed to recover from post-weaning stress (Wijtten et al., 2011; Yang et al., 2016).

The improved ATTD of dry matter, crude protein, IDF, TDF, and GE, and greater concentration of DE in response to addition of xylanase or stimbiotic to the diets is in agreement with data indicating that addition of xylanase to diets for broiler chickens or pigs increased digestibility of dry matter, crude protein, energy, and non-starch polysaccharides (Kiarie et al., 2014; Lan et al., 2017, Duarte et al., 2019; Chen et al., 2020; Craig et al., 2020, Moita et al., 2022). Xylanase hydrolyzes the xylose backbone of arabinoxylans, which may result not only in greater solubilization of insoluble dietary fiber and thus available to fermentation, but also in the release of trapped nutrients, and reduced digesta viscosity, leading to an increase in nutrient and energy digestibility (De Lange et al., 2010; Adeola and Cowerson, 2011; De Vries et al., 2012;

Zhang et al., 2014; González-Ortiz et al., 2016; Raza et al., 2019). However, it has not been demonstrated that changes in viscosity due to addition of xylanase to diets for pigs have any impact on nutrient digestibility (Duarte et al., 2019). Likewise, XOS are sugar oligomers (2 to 10 monomeric units), which may act as prebiotics in the diet for pigs, modifying the microbial ecology (Gibson and Roberfroid, 1995; McLoughlin et al., 2017), resulting in increased activity of beneficial gut bacteria such as *Bifidobacterium* spp. (Finegold et al., 2014), reduced pH in the hindgut, and increased synthesis of short-chain fatty acids that decrease intestinal pathogens and provide energy to the host (Smiricky-Tjardes et al., 2003; Bedford and Cowierson, 2012; Pan et al., 2019; Tiwari et al., 2020). Because of the modes of action of xylanases and stimbiotics, these additives are believed to be nutrient enhancing compounds that have the overall effects of increasing energy absorption by pigs. Results of the experiment demonstrating the increased ATTD of fiber and energy support this hypothesis. Improvements in digestibility of dry matter, protein, energy, and concentrations of DE observed during phases 2 and 3 as xylanase or stimbiotic was included in the diets resulted in an improvement in growth performance, which is likely primarily due to increased hydrolysis of insoluble dietary fiber. It is possible that the combination of the mechanisms of action of xylanase and XOS increased the efficiency of fermenting dietary fiber as indicated by the greater effect of adding stimbiotic than xylanase to the phase-2 diets. As a consequence, the hypothesis that xylanase and stimbiotic improve growth performance and energy and fiber digestibility when included in diets for weanlings pigs was accepted.

The observation that sow treatment had no impact on growth performance or nutrient digestibility by the offspring indicates that there is no carry over effect from sows to the offspring. This may indicate that xylanase in sow diets does not change the microbial

composition of sow feces, but because we did not determine microbial composition, we cannot confirm this hypothesis.

Diarrhea incidence and markers of intestinal health

The observed increase in diarrhea incidence for pigs fed xylanase in phase 3 is in contrast with data indicating that xylanase reduced diarrhea rate or did not impact diarrhea (Lan et al., 2017; Sutton et al., 2021; Boontiam et al., 2022), and XOS reduced diarrhea rate compared with xylanase when fed to pigs (Bai et al., 2021; Wang et al., 2023). The type of dietary fiber (soluble vs. insoluble) may influence the physicochemical properties of the digesta and the intestinal microbiota (Canibe and Bach Knudsen, 2002), which may be impacted by xylanase or stimbiotic supplementation. More research needs to be conducted to clarify the effects of xylanase and stimbiotic on diarrhea in weanling pigs. However, it is possible that xylanase resulted in some insoluble fiber becoming solubilized, and if these soluble fibers were not fully fermented in the hindgut, they may have resulted in higher moisture content and loss of fecal consistency.

Low plasma urea N is an indicator of efficient amino acid utilization (Kohn et al., 2005). The observation that pigs fed the diet with xylanase tended to have reduced plasma urea N was in agreement with decreased plasma urea N concentration when xylanase was fed to weanling pigs (Lan et al., 2017). Total protein and albumin are indicators of transport of nutrients in the blood (Bern et al., 2015), and the increased total protein and albumin observed in pigs fed diets containing stimbiotic in phase 2, therefore, indicates that nutrient transport was more efficient, which may have supported the increased growth performance.

Peptide YY and GIP are gastrointestinal hormones that are important in the regulation of feed intake, energy homeostasis, gastric acid secretion, and gastrointestinal motility, and their levels increase immediately after nutrient ingestion (Ueno, 2008; Vella, 2015). It was

hypothesized that PYY and GIP would be impacted by xylanase and stimbiotic, as previously reported (Chen et al., 2020; Casas and Stein, 2016), but the lack of effects of both xylanase and stimbiotic on stimulating PYY and GIP indicates that factors other than those determined in this experiment influence plasma concentrations of PYY and GIP.

Fibrous ingredients fed to weanling pigs may increase oxidative stress and intestinal inflammation due to physicochemical characteristics resulting in increased digesta viscosity, and greater pathogenic load (Tiwari et al., 2018). Inflammation is regulated by the immune system by innate and adaptive responses, which produce cytokines with pro-inflammatory or anti-inflammatory effects (Murphy and Weaber, 2017). The reduction in the pro-inflammatory cytokines IL-1α and IL-2 and the anti-inflammatory cytokines IL-4 and IL-10 indicates a positive effect of xylanase on the immune response of pigs, possibly due to alteration of the intestinal microbiota composition. The lack of an effect of stimbiotic on concentrations of cytokines is in contrast with results of research indicating that there are anti-inflammatory effects of stimbiotic in pigs and broilers (Hou et al., 2020; Yuan et al., 2018). This discrepancy may suggest that stimbiotic may interact with the gut microbiota differently than xylanase alone, but we cannot confirm this hypothesis because we did not determine microbial composition. The impact of xylanase and stimbiotic on immune response may also depend on the existing microbial community (Song et al., 2023), which can vary among environmental conditions, pig genetics, health status, and diet composition. The potential mechanism of xylanase and stimbiotic associated with the reduction in inflammatory response parameters remains unclear and research to elucidate effects of xylanase and stimbiotic on regulation of cytokines is needed.

An improved nutrient digestibility in diets containing xylanase or stimbiotic may result from changes in intestinal morphology because intestinal villi are the site of nutrient absorption

Duarte et al., 2019; 2020; Chen et al., 2020). However, the lack of any impact of xylanase or stimbiotic on intestinal morphology, which has also been observed previously (Liu et al., 2018; He et al., 2020), indicates that the increased ATTD of energy and nutrients that was observed for pigs fed xylanase or stimbiotic was unrelated to changes in intestinal morphology.

The permeable barrier of the intestine may be modified by xylanase or stimbiotic added to diets for pigs, resulting in increased relative mRNA abundance of tight junction proteins (i.e., occludin and zonula occludens-1) in the jejunum and ileal mucosa of pigs [Tiwari et al., 2018; Yin et al., 2019; Chen et al., 2020; He et al., 2020]. However, the observation that xylanase or stimbiotic did not impact the mRNA abundance of tight junction proteins in this experiment, despite greater nutrient digestibility, indicates that there may be other interactions among diet, mucosa, and the intestinal microbiota that may impact intestinal integrity.

CONCLUSIONS

Feeding diets with xylanase or stimbiotic to weanling pigs increased nutrient digestibility in the late nursery, leading to greater growth performance after 42 d post-weaning; however, feeding sows xylanase in lactation did not influence pig growth performance after weaning. Although supplementation of a stimbiotic increased nutrient transport proteins in the blood, and xylanase led to positive immune responses by decreasing cytokines related to inflammation, xylanase or stimbiotic did not impact intestinal morphology and tight junction protein expression, which suggests that the mechanisms underlying improved digestibility and performance of weanling pigs may be more complex and involve interactions among diet, host and gut microbiota, physiology, and immune function. Additional research is needed to elucidate more biological mechanisms and interactions between host metabolism, fermentation by

microbial populations, and xylanase, XOS, and stimbiotic alone or in combination in diets for weanling pigs.

TABLES

Table 6.1. Analyzed nutrient composition main of ingredients in diets (as-fed basis)

Item	Corn	Soybean meal	Wheat middlings	Fermented soybean meal[1]	Fish meal
Gross energy, kcal/kg	3,816	4,133	3,997	4,213	4,320
Dry matter, %	85.85	89.25	88.88	87.63	94.93
Ash, %	1.33	6.12	5.99	6.69	20.82
Acid hydrolyzed ether extract, %	3.70	3.30	4.84	0.75	10.15
Crude protein, %	6.58	47.25	15.37	49.1	64.03
Starch, %	65.40	1.27	19.70	1.35	-
Insoluble dietary fiber, %	9.20	14.30	38.40	15.90	3.20
Soluble dietary fiber, %	0.70	3.70	3.20	3.70	1.30
Total dietary fiber, %	9.90	18.00	41.60	19.60	4.50
Indispensable amino acids, %					
Arg	0.31	3.29	1.05	3.14	3.67
His	0.19	1.22	0.42	1.21	1.33
Ile	0.26	2.21	0.51	2.42	2.53
Leu	0.76	3.61	0.91	3.74	4.17
Lys	0.24	2.95	0.65	2.92	4.79
Met	0.13	0.65	0.21	0.65	1.63
Phe	0.32	2.41	0.58	2.50	2.33

Table 6.1. (cont.)

Thr	0.23	1.82	0.47	1.87	2.44
Trp	0.04	0.55	0.15	0.66	0.52
Val	0.32	2.37	0.72	2.46	2.89
Dispensable amino acids, %					
Ala	0.48	2.03	0.71	2.16	3.90
Asp	0.44	5.21	1.06	5.46	5.44
Cys	0.15	0.66	0.33	0.67	0.49
Glu	1.21	8.57	2.74	8.35	8.03
Gly	0.27	1.95	0.81	2.06	4.74
Pro	0.56	2.28	0.90	2.44	2.98
Ser	0.29	2.04	0.54	2.07	2.09
Tyr	0.20	1.68	0.37	1.70	1.75
Minerals					
Ca, %	0.02	0.33	0.20	0.33	5.39
P, %	0.25	0.71	0.99	0.75	3.53
K, %	0.30	2.61	1.13	2.29	1.14
Mg, %	0.07	0.33	0.40	0.29	0.25
Na, %	0.01	0.15	0.02	0.01	0.77
Cu, mg/kg	6.87	43.50	34.05	37.94	66.11
Fe, mg/kg	33.45	210.86	154.52	123.22	1058.61
Mn, mg/kg	12.56	86.90	169.01	76.54	177.22
Zn, mg/kg	25.21	895.70	106.68	71.26	177.41

Table 6.1. (cont.)

[1]Fermented soybean meal (Industrial De Oleaginosas Americanas S.A., Barranca, Costa Rica).

Table 6.2. Ingredient composition of experimental diets, as-fed basis

	Control diets[1]		
Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	41.78	47.96	61.82
Soybean meal	20.00	26.00	25.00
Wheat middlings	2.50	5.00	7.50
Fermented soybean meal[2]	10.00	7.50	-
Lactose	15.00	7.50	-
Fish meal	5.00	-	-
Soybean oil	2.18	2.18	2.00
Limestone	0.80	1.00	0.94
Dicalcium phosphate	0.70	0.90	0.87
L-Lys HCl	0.45	0.40	0.37
DL-Met	0.16	0.15	0.10
L-Thr	0.13	0.11	0.10
Titanium dioxide	0.40	0.40	0.40
Salt	0.40	0.40	0.40
Vitamin-mineral premix[3]	0.50	0.50	0.50

[1]Two additional diets were formulated by adding a 1% premix with xylanase or a 1% premix with stimbiotic (i.e. combination of xylanase and xylo-oligosaccharides) to the control diet in each phase.

The premix with xylanase contained 1.6 million BXU/kg of exogenous xylanase (0.1 kg xylanase concentrate containing 160 million BXU/kg was mixed with 9.9 kg of ground corn).

Table 6.2. (cont.)

The premix with stimbiotic contained 1.6 million BXU/kg of exogenous xylanase (0.1 kg xylanase plus xylo-oligosaccharides concentrate containing 160 million BXU/kg was mixed with 9.9 kg of ground corn). At 1% inclusion, the diet with xylanase and the diet with stimbiotic were expected to contain 16,000 BXU/kg of xylanase. BXU is the amount of enzyme that will release 0.06 micromoles of reducing sugars (xylose equivalents) from beechwood xylan per min at pH 5.3 and 50 ºC.

[2]Fermented soybean meal (Industrial De Oleaginosas Americanas S.A., Barranca, Costa Rica).

[3]The vitamin-micromineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

Table 6.3. Analyzed nutrient composition of experimental diets (as-fed basis)

Item	Phase 1			Phase 2			Phase 3		
	Control	Xylanase[1]	Stimbiotic[2]	Control	Xylanase	Stimbiotic	Control	Xylanase	Stimbiotic
GE[3], kcal/kg	3,997	3,997	3,982	3,881	3,892	3,907	3,833	3,849	3,887
Dry matter, %	89.66	89.61	89.40	86.80	87.45	87.22	86.52	86.30	87.03
Ash, %	6.00	6.11	5.84	4.59	4.80	5.82	5.15	5.52	5.16
AEE[3], %	4.21	3.80	3.88	3.10	2.93	3.02	4.59	4.71	5.55
CP[3], %	20.33	20.66	20.54	17.10	17.45	17.99	15.91	16.03	16.37
Starch, %	29.59	29.20	29.34	38.34	37.47	36.27	40.75	40.40	40.83
IDF[3], %	9.90	10.10	9.90	11.40	11.30	11.10	12.80	12.80	12.70
SDF[3], %	3.30	3.40	3.30	2.80	2.50	2.30	1.90	1.80	1.40
TDF[3], %	13.20	13.50	13.20	14.20	13.80	13.40	14.70	14.60	14.10
Arg	1.25	1.23	1.26	1.08	1.12	1.21	1.00	1.03	1.04
His	0.52	0.50	0.52	0.45	0.45	0.46	0.42	0.42	0.43
Ile	0.95	0.91	0.96	0.79	0.79	0.80	0.71	0.69	0.70

Table 6.3. (cont.)

Leu	1.68	1.62	1.68	1.46	1.46	1.51	1.40	1.36	1.38
Lys	1.54	1.53	1.55	1.22	1.21	1.30	1.09	1.10	1.17
Met	0.50	0.49	0.50	0.35	0.37	0.40	0.31	0.32	0.35
Phe	0.99	0.96	1.00	0.86	0.86	0.88	0.79	0.78	0.80
Thr	0.84	0.87	0.88	0.70	0.74	0.75	0.65	0.68	0.71
Trp	0.23	0.22	0.22	0.17	0.18	0.20	0.15	0.17	0.16
Val	0.98	0.98	0.98	0.85	0.84	0.90	0.79	0.76	0.77
Dispensable amino acids, %									
Ala	1.04	1.01	1.00	0.84	0.86	0.85	0.83	0.82	0.82
Asp	2.07	2.03	1.90	1.74	1.78	1.90	1.56	1.58	1.62
Cys	0.31	0.29	0.26	0.28	0.28	0.30	0.26	0.27	0.28
Glu	3.49	3.43	3.30	3.05	3.14	3.40	2.87	2.87	2.94
Gly	0.94	0.92	0.91	0.71	0.73	0.74	0.67	0.68	0.68
Pro	1.17	1.11	1.03	1.04	1.01	1.00	0.99	0.94	0.97
Ser	0.80	0.82	0.75	0.69	0.76	0.78	0.66	0.71	0.73

Table 6.3. (cont.)

Tyr	0.64	0.63	0.60	0.55	0.57	0.56	0.49	0.52	0.53
Minerals									
Ca, %	2.27	1.92	2.14	1.37	1.01	1.33	1.03	0.92	0.94
P, %	1.40	1.34	1.57	1.11	1.12	1.09	0.71	0.76	0.87
K, %	1.71	1.59	1.78	1.54	1.80	1.66	0.95	0.95	1.13
Mg, %	0.31	0.28	0.31	0.29	0.33	0.29	0.21	0.20	0.23
Na, %	0.39	0.37	0.42	0.23	0.25	0.27	0.17	0.18	0.23
Cu, mg/kg	197.81	120.34	107.43	90.52	92.54	89.55	64.76	60.87	66.59
Fe, mg/kg	410.31	381.84	437.13	312.18	332.70	352.69	225.82	225.53	227.05
Mn, mg/kg	228.78	207.76	227.25	181.26	206.79	199.30	152.33	146.98	164.10
Zn, mg/kg	469.96	502.10	481.37	279.58	310.95	296.05	187.09	185.42	275.31
XU[4]	<2,000	13,700	16,600	<2,000	15,000	23,700	<2,000	13,900	17,800

[1]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK.

[2]Stimbiotic = Signis; AB Vista, Marlborough, UK.

[3]GE = gross energy; AEE = Acid-hydrolyzed ether extract; CP = crude protein; IDF = insoluble dietary fiber; SDF = soluble dietary fiber; TDF = total dietary fiber.

Table 6.3. (cont.)

[4]Xylanase units, BXU/kg. BXU is the amount of enzyme that will release 0.06 micromoles of reducing sugars (xylose equivalents) from beechwood xylan per min at pH 5.3 and 50 ºC. None of the control diets exceeded the detection limit (2,000 BXU/kg).

Table 6.4. Forward and reverse primer sequences used for the quantitative reverse transcription-polymerase chain reaction

Item	Primer sequences (5'→3')		Reference
	Forward	Reverse	
Internal control genes			
Hypoxanthine-guanine phosphoribosyl transferase	GGACTTGAATCATGTTTGTG	CAGATGTTTCCAAACTCAAC	Gonzalez et al. (2013)
Glyceraldehyde 3-phosphate dehydrogenase	ATCCTGGGCTACACTGAGGAC	AAGTGGTCGTTGAGGGCAATG	Nygard et al. (2007)
Gut-protective target genes			
Occludin	TCCTGGGTGTGATGGTGTTC	CGTAGAGTCCAGTCACCGCA	Hu et al. (2013)
Zonula occludens-1	AAGCCCTAAGTTCAATCACAATCT	ATCAAACTCAGGAGGCGGC	Hu et al. (2013)

Table 6.5. Growth performance of pigs[1, 2]

Item	Sow treatment				Post-weaning dietary treatment				
	Control	Xylanase[3]	SEM	*P*-value	Control	Xylanase	Stimbiotic[3]	SEM	*P*-value
Phase 1 (d 1 to 14)									
Initial body weight, kg	5.77	5.84	0.20	0.589	5.81	5.83	5.77	0.21	0.924
ADG[4], g	97.44	83.95	10.79	0.196	102.40	85.17	84.54	11.65	0.235
ADFI[4], g	171.90	171.60	8.28	0.979	182.10	165.30	167.90	8.28	0.294
G:F[4]	0.54	0.49	0.05	0.187	0.56	0.50	0.50	0.06	0.308
Final body weight, kg	7.13	7.02	0.24	0.555	7.25	7.02	6.96	0.26	0.395
Phase 2 (d 15 to 28)									
ADG, g	430.60	440.50	15.54	0.542	391.70^{b}	434.70^{b}	480.20^{a}	16.99	<0.001
ADFI, g	654.20	666.40	22.11	0.632	652.10	663.80	665.10	24.60	0.885
G:F	0.66	0.66	0.01	0.867	0.60^{c}	0.66^{b}	0.73^{a}	0.01	<0.001
Final body weight, g	13.17	13.18	0.45	0.973	12.73^{y}	13.11^{xy}	13.68^{x}	0.47	0.073
Phase 3 (d 29 to 42)									
ADG, g	618.10	626.10	14.95	0.701	566.80^{b}	648.30^{a}	651.20^{a}	17.25	0.001

Table 6.5. (cont.)

ADFI, g	1054.10	1037.40	26.90	0.577	1001.10[y]	1071.40[xy]	1064.60[x]	29.73	0.084
G:F	0.59	0.61	0.02	0.310	0.57[y]	0.61[x]	0.62[x]	0.02	0.098
Final body weight, kg	21.78	21.99	0.45	0.697	20.67[b]	22.18[a]	22.80[a]	0.50	0.004
Overall (d 1 to 42)									
ADG, g	381.70	383.90	8.72	0.851	353.60[b]	389.40[a]	405.30[a]	10.04	0.001
ADFI, g	626.10	625.80	11.19	0.983	611.80	633.50	632.50	13.71	0.455
G:F	0.61	0.62	0.02	0.441	0.58[b]	0.61[a]	0.64[a]	0.02	<0.001

[a-c]Within columns in the post-weaning dietary treatment main effect, values within a row lacking a common superscript differ ($P < 0.05$)

[x-y]Within columns in the post-weaning dietary treatment main effect, values within a row lacking a common superscript tend to be different ($P < 0.10$)

[1]Data are least square means of 24 observations per sow treatment, and 16 observations per post-weaning dietary treatment.

[2]There was no interaction between sow treatment and post-weaning dietary treatment for any of the growth performance parameters, therefore, the interaction term was removed from the final model and only main effects are presented.

[3]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Stimbiotic = Signis; AB Vista, Marlborough, UK.

[4]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

Table 6.6. Average diarrhea scores and incidence of diarrhea in pigs[1, 2]

	Sow treatment				Post-weaning dietary treatment				
Item	Control	Xylanase[3]	SEM	*P*-value	Control	Xylanase	Stimbiotic[3]	SEM	*P*-value
Diarrhea score[4]									
d 1 to 14 (Phase 1)	2.30	2.23	0.24	0.551	2.37	2.13	2.30	0.25	0.192
d 15 to 28 (Phase 2)	2.34	2.33	0.13	0.935	2.18	2.39	2.43	0.14	0.188
d 29 to 42 (Phase 3)	1.42	1.58	0.07	0.090	1.26[b]	1.71[a]	1.54[a]	0.08	0.001
d 1 to 42 (Overall Phase)	2.02	2.05	0.11	0.711	1.93	2.07	2.09	0.12	0.202
Frequency of diarrhea									
d 1 to 14 (Phase 1)									
Pen d[5]	168	168	-	-	112	112	112	-	-
Frequency[6]	39.88	42.86	-	0.580	48.21	33.93	41.96	-	0.094
d 15 to 28 (Phase 2)									
Pen d	168	168	-	-	112	112	112	-	-
Frequency	38.10	41.67	-	0.504	31.25	41.96	46.43	-	0.058
d 29 to 42 (Phase 3)									

Table 6.6. (cont.)

Pen d	168	168	-	-	112	112	112	-	-
Frequency	6.55	11.31	-	0.126	1.79[c]	16.96[a]	8.04[b]	-	<0.001
d 1 to 42 (Overall phase)									
Pen d	504	504	-	-	336	336	336	-	-
Frequency	28.14	31.94	-	0.192	27.08	30.95	32.14	-	0.327

[a-b]Within columns in the post-weaning dietary treatment main effect, values within a row lacking a common superscript differ ($P < 0.05$)

[1]Data are least square means of 24 observations per sow treatment, and 16 observations per post-weaning dietary treatment.

[2]There was no interaction between sow treatment and post-weaning dietary treatment for any of the diarrhea scores and incidence of diarrhea parameters, therefore, the interaction term was removed from the final model and only main effects are presented.

[3]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Stimbiotic = Signis; AB Vista, Marlborough, UK.

[4]Fecal scores: 1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea.

[5]Pen d = number of pens x number of days assessing diarrhea scores.

[6]Frequency = (number of pen days with diarrhea scores ≥ 3 divided by pen days) × 100.

Table 6.7. Apparent total tract digestibility (ATTD) of nutrients and digestible energy by pigs[1, 2]

	Sow treatment				Post-weaning dietary treatment				
Item	Control	Xylanase[3]	SEM	*P*-value	Control	Xylanase	Stimbiotic[3]	SEM	*P*-value
Phase 2, d 26 to 28									
ATTD of dry matter, %	79.22	78.49	0.72	0.398	76.45[b]	78.42[b]	81.69[a]	0.81	<0.001
ATTD of gross energy, %	77.83	76.74	0.84	0.260	74.50[b]	76.80[b]	80.55[a]	0.93	<0.001
Digestible energy, kcal/kg	3,030	2,988	32.68	0.260	2,901[b]	2,990[b]	3,136[a]	32.68	<0.001
ATTD of starch, %	98.28	98.10	0.19	0.510	97.83	98.18	98.56	0.24	0.103
ATTD of crude protein, %	74.67	73.60	1.29	0.391	71.64[b]	73.65[b]	77.11[a]	1.39	0.001
ATTD of IDF[4], %	43.92	41.24	2.34	0.284	35.76[b]	41.24[b]	50.75[a]	2.56	<0.001
ATTD of SDF[4], %	76.39	79.66	1.97	0.211	75.40	77.88	80.80	2.25	0.192
ATTD of TDF[4], %	49.89	48.28	1.99	0.451	43.04[b]	47.96[b]	56.26[a]	2.18	<0.001
Phase 3, d 40 to 42									
ATTD of dry matter, %	79.57	78.97	0.53	0.376	77.70[b]	79.69[a]	80.42[a]	0.60	0.002
ATTD of gross energy, %	78.26	77.64	0.56	0.388	76.16[b]	78.53[a]	79.15[a]	0.64	0.001
Digestible energy, kcal/kg	3,018	2,994	21.63	0.388	2,937[b]	3,028[a]	3,052[a]	24.51	0.001

Table 6.7. (cont.)

ATTD of starch, %	98.55	98.48	0.14	0.727	98.30	98.66	98.59	0.17	0.286
ATTD of crude protein, %	74.70	73.24	0.75	0.173	72.32[b]	73.94[ab]	75.65[a]	0.88	0.030
ATTD of IDF, %	49.39	47.28	1.56	0.243	44.06[b]	49.63[a]	51.32[a]	1.74	0.002
ATTD of SDF, %	66.35	67.33	4.60	0.807	67.06	66.61	66.85	4.90	0.995
ATTD of TDF, %	51.48	49.55	1.80	0.246	46.76[b]	51.63[a]	53.15[a]	1.93	0.004

[a-b]Within columns in the post-weaning dietary treatment main effect, values within a row lacking a common superscript differ ($P < 0.05$)

[1]Data are least square means of 24 observations per sow treatment, and 16 observations per post-weaning dietary treatment.

[2]There was no interaction between sow treatment and post-weaning dietary treatment for any of the digestibility parameters, therefore, the interaction term was removed from the final model and only main effects are presented.

[3]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Stimbiotic = Signis; AB Vista, Marlborough, UK.

[4]IDF = insoluble dietary fiber; SDF = soluble dietary fiber; TDF = total dietary fiber.

Table 6.8. Plasma characteristics and cytokines of pigs, d 28[1, 2]

	Sow treatment				Post-weaning dietary treatment				
Item	Control	Xylanase[3]	SEM	*P*-value	Control	Xylanase	Stimbiotic[3]	SEM	*P*-value
Plasma urea N, mg/dL	6.00	5.96	0.42	0.944	6.25^{xy}	5.00^{y}	6.69^{x}	0.51	0.062
Total protein, g/dL	5.00	4.81	0.04	0.002	4.81^{b}	4.89^{ab}	5.02^{a}	0.05	0.020
Albumin, g/dL	2.90	2.88	0.08	0.805	2.77^{b}	2.91^{ab}	2.99^{a}	0.08	0.012
Peptide YY, ng/mL	1.24	1.35	0.06	0.162	1.31	1.31	1.27	0.07	0.914
GIP[4], pg/mL	788.50	839.53	68.61	0.545	797.26	741.32	903.47	75.25	0.203
Cytokine, ng/mL									
IFNγ[4]	4.62	4.85	1.04	0.796	5.86	3.61	4.75	1.12	0.123
IL[4]-1α	0.02	0.02	0.01	0.333	0.02^{a}	0.01^{b}	0.03^{a}	0.01	0.011
IL-1β	0.10	0.09	0.01	0.345	0.11^{x}	0.06^{y}	0.11^{x}	0.02	0.051
IL-1RA	0.28	0.18	0.02	0.004	0.24	0.19	0.26	0.03	0.185
IL-2	0.09	0.07	0.02	0.663	0.11^{a}	0.03^{b}	0.10^{a}	0.02	0.027
IL-4	0.25	0.23	0.06	0.862	0.34^{a}	0.07^{b}	0.31^{a}	0.07	0.007
IL-6	0.05	0.03	0.01	0.200	0.05	0.04	0.04	0.01	0.765

Table 6.8. (cont.)

IL-8	0.02	0.01	0.00	0.054	0.01	0.02	0.01	0.00	0.513
IL-10	0.32	0.29	0.08	0.672	0.37[a]	0.13[b]	0.41[a]	0.09	0.010
IL-12	0.78	0.74	0.05	0.640	0.71	0.86	0.71	0.06	0.133
IL-18	0.98	0.78	0.13	0.283	0.89	0.77	0.99	0.16	0.638
TNFα[4]	0.05	0.03	0.01	0.103	0.05	0.04	0.04	0.01	0.362

[a-b]Within columns in the post-weaning dietary treatment main effect, values within a row lacking a common superscript differ ($P < 0.05$)

[x-y]Within columns in the post-weaning dietary treatment main effect, values within a row lacking a common superscript tend to be different ($P < 0.10$)

[1]Data are least square means for each dependent variable represent 18 to 24 observations per sow treatment, and 13 to 16 observations per post-weaning dietary treatment.

[2]There was no interaction between sow treatment and post-weaning dietary treatment for any of the plasma and cytokines parameters, therefore, the interaction term was removed from the final model and only main effects are presented.

[3]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Stimbiotic = Signis; AB Vista, Marlborough, UK.

[4]GIP = Gastric inhibitory polypeptide; IFNγ = interferon-γ; IL = interleukin; TNFα = tumor necrosis factor-α.

Table 6.9. Intestinal tissue morphology of pigs, d 14[1, 2]

	Sow treatment				Post-weaning dietary treatment				
Item	Control	Xylanase[3]	SEM	P-value	Control	Xylanase	Stimbiotic[3]	SEM	P-value
Ileum, um									
Villus Height	321.43	314.52	17.17	0.568	329.02	305.47	319.43	17.92	0.229
Crypt Depth	238.77	237.84	12.54	0.903	241.16	234.93	238.81	12.98	0.769
Lamina propria thickness	50.18	49.11	1.13	0.509	48.46	50.83	49.64	1.39	0.493
VH:CD[4]	1.33	1.33	0.02	0.937	1.36	1.31	1.32	0.03	0.370

[1]Data are least square means for each dependent variable represent 20 to 24 observations per sow treatment, and 14 to 16 observations per post-weaning dietary treatment.

[2]There was no interaction between sow treatment and post-weaning dietary treatment for any of the morphology parameters, therefore, the interaction term was removed from the final model and only main effects are shown.

[3]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Stimbiotic = Signis; AB Vista, Marlborough, UK.

[4]VH:CD = villus height: crypt depth ratio.

Table 6.10. Least squares means (log2-backtransformed) for mRNA abundance of genes in the ileum of pigs, d 14[1, 2]

	Sow treatment				Post-weaning dietary treatment				
Item	Control	Xylanase[3]	SEM	P-value	Control	Xylanase	Stimbiotic[3]	SEM	P-value
Occludin	1.10	0.88	1.12	0.147	1.00	0.90	1.06	1.14	0.666
Zonula occludens-1	2.13	1.91	1.12	0.443	2.13	2.03	1.89	1.13	0.773

[1]Data are least square means for each dependent variable represent 20 to 24 observations per sow treatment, and 14 to 16 observations per post-weaning dietary treatment.

[2]There was no interaction between sow treatment and post-weaning dietary treatment for any of the gene expression parameters, therefore, the interaction term was removed from the final model and only main effects are presented.

[3]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Stimbiotic = Signis; AB Vista, Marlborough, UK

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CHAPTER 7: EFFECTS OF XYLANASE ALONE OR IN COMBINATION WITH XYLO-OLIGOSACCHARIDES ON APPARENT ILEAL DIGESTIBILITY, APPARENT CECAL DIGESTIBILITY, AND APPARENT TOTAL TRACT DIGESTIBILITY OF ENERGY AND DIETARY FIBER IN DIETS CONTAINING WHEAT MIDDLINGS FED TO GROWING PIGS

ABSTRACT

The hypothesis that xylanase alone, and xylanase and xylo-oligosaccharides (**XOS**) in combination can improve apparent ileal digestibility (**AID**), apparent cecal digestibility (**ACD**), and apparent total tract digestibility (**ATTD**) of gross energy, insoluble dietary fiber (**IDF**), soluble dietary fiber (**SDF**), and total dietary fiber (**TDF**) in diets containing wheat middlings fed to growing pigs was tested. Twenty-four barrows (initial body weight: 48.39 ± 7.30 kg) with a T-cannula in the distal ileum and another T-cannula in the proximal colon were allotted to a randomized complete block design with 8 pigs per diet and body weight was the blocking factor. The control diet was based on corn, soybean meal and wheat middlings, and two additional diets were formulated by supplementing the control diet with 100 g/ton of xylanase, or 100 g/ton of xylanase and 50 g/ton of XOS. A blood sample was collected after following a 27-d adaptation period and plasma was analyzed for peptide YY. Fecal samples were collected from d 28 to d 31, colon digesta samples were collected on d 32 and 33, and ileal digesta samples were collected on d 34 and 35. Results indicated that the AID of dry matter, gross energy, IDF, and TDF was greater ($P < 0.05$) in the diet containing xylanase and in the diet containing xylanase and XOS compared with the control diet, but the diet containing xylanase had greater ($P < 0.05$) ACD and ATTD of dry matter, gross energy, IDF, and TDF compared with the control diet. However, peptide YY concentrations in the plasma of pigs was not impacted by xylanase or xylanase and

XOS addition to the diet. In conclusion, addition of xylanase to a diet fed to growing pigs increased the AID, ACD, and ATTD of dry matter, gross energy, IDF, and TDF, and xylanase and XOS increased AID of dry matter, gross energy, IDF, and TDF in diets, but xylanase and XOS did not increase ACD and ATTD of nutrients and energy in diets.

Keywords: digestibility, energy, fiber, pigs, xylanase, xylo-oligosaccharides

Abbreviations: ACD, apparent cecal digestibility; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; AXOS, arabinoxylo-oligosaccharides; BXU, beechwood xylanase units; FTU, phytase units; IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber; XOS, xylo-oligosaccharides.

INTRODUCTION

Inclusion of wheat middlings in diets for pigs has increased in recent decades because it is a less expensive source of nutrients compared with cereal grains (Stein et al., 2016; Stas et al., 2024). However, increasing inclusion of wheat middlings in diets for pigs can reduce growth performance of pigs (De Jong et al., 2014; Du et al., 2023) due to reduced digestibility of organic matter and energy compared with diets containing corn or wheat (Casas et al., 2018; Espinosa et al., 2024; Son et al., 2023). Wheat middlings contain more total dietary fiber (**TDF**) than wheat, as the concentration of fiber from the pericarp and aleurone layer are concentrated when the starch in the endosperm is extracted during processing (Rosenfelder et al., 2013). Arabinoxylans is the main type of dietary fiber in cereal grains and cereal grain coproducts, and represent 60 to 70% of the TDF in wheat middlings (Jaworski et al., 2015; Barron et al., 2020; Wang et al.,

2020). Arabinoxylans are polysaccharides made up of a chain of xylose units, that in wheat middlings is characterized by a complex side-chain structure of arabinose substitutions, with an arabinose/xylose ratio between 0.6 and 1.0, and with substitutions of glucuronic acid and phenolic acid (Kalathunga and Islam, 2025). Arabinoxylans are mostly insoluble because they can form cross-linkages with other cell wall components, including lignin, making arabinoxylans undigestible by pigs and hardly fermentable by microbes (Marcotuli et al., 2015; Chen et al., 2024). However, xylanase can hydrolyze the β-(1− 4) glycosidic bonds in the xylan backbone of arabinoxylans, resulting in the production of arabinoxylo-oligosaccharides (**AXOS**) that are soluble and therefore, fermentable by the intestinal microbes in the hindgut resulting in synthesis of short-chain fatty acids that can be used as a source of energy by pigs (Abelilla and Stein, 2019; Petry and Patience, 2020). Likewise, xylo-oligosaccharides (**XOS**), which consist of unsubstituted chains of 2 to 10 xylose units linked by β(1–4) bonds, are industrially produced molecules that can be added in diets for pigs at low inclusion rates (Singh et al., 2015; Gonzalez-Ortiz et al., 2019). Xylo-oligosaccharides may promote fiber utilization by signaling fiber-fermenting bacteria to increase their activity, thereby promoting synthesis of short-chain fatty acids, acting as a stimbiotic (Gonzalez-Ortiz et al., 2019; Cho et al., 2020). However, the mechanisms of action of AXOS and XOS related to the fermentation in the gastrointestinal tract of pigs are not fully understood. Although greater stimulation of fermentation that is initiated by xylanase or XOS may result in greater proportion of energy from short-chain fatty acids (Bach Knudsen, 2011; Tiwari et al., 2018; Chen et al., 2021; González-Solé et al., 2022), it is unknown if the effect of XOS in combination with AXOS produced by xylanase is similar to that of AXOS alone. It is believed that a significant part of fiber fermentation occurs in the cecum of pigs, but the extent of the fermentation stimulated by xylanase or the combination of xylanase and XOS

on dietary fiber fractions in different parts of the intestinal tract has not been reported. Therefore, an experiment was conducted to test the hypothesis that xylanase and xylanase and XOS in combination may improve apparent ileal digestibility (**AID**), apparent cecal digestibility (**ACD**), and apparent total tract digestibility (**ATTD**) of gross energy, insoluble dietary fiber (**IDF**), soluble dietary fiber (**SDF**), and TDF by growing pigs in diets containing wheat middlings.

MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was reviewed and approved prior to initiation of the experiment. Pigs used in this experiment were the offspring of Camborough sows mated to Line 800 boars (Pig Improvement Company, Hendersonville, TN, USA).

Experimental diets

A corn-soybean meal-wheat middlings control diet was formulated to meet nutrient requirements of growing pigs from 60 to 90 kg (NRC, 2012; Table 7.1). Two additional diets were formulated by supplementing the control diet with 100 g/ton of xylanase, or 100 g/ton of xylanase and 50 g/ton of a XOS. The exogenous xylanase (Econase XT 25; AB Vista, Marlborough, UK) and the XOS (AB Vista, Marlborough, UK) were included in diets as recommended by the supplier. The 100 g/t of xylanase in the diets was expected to provide 16,000 beechwood xylanase units (**BXU**) per kg of diet. One BXU is defined as the amount of enzyme that will release 0.06 μmoles of reducing sugars (xylose equivalents) from beechwood xylan per min at pH 5.3 and 50 ºC. Thus, a total of 3 diets were used (Tables 7.2 and 7.3). All diets were also supplemented with 500 phytase units (**FTU**)/kg of microbial phytase (Quantum-Blue 5G; AB Vista, Marlborough, UK). Vitamins and minerals were included in all diets to meet

or exceed the estimated nutrient requirements for growing pigs (NRC, 2012). All diets also contained 0.50% titanium dioxide as an indigestible marker.

Animals, housing, and experimental design

Twenty-four growing barrows (initial body weight: 48.39 ± 7.30 kg) that were prepared with a T-cannula in the distal ileum and a second T-cannula in the anterior part of the colon right after the cecum (Stein et al., 1998; Jaworski and Stein, 2017). Pigs had their cannulas installed when they were 26.57 ± 2.53 kg and had been used in a previous experiment before being allotted to the three diets in this experiment. Pigs were allotted to a randomized complete block design with 8 pigs per diet and body weight was the blocking factor. Pigs were housed in pens (1.2 × 1.5 m) in an environmentally controlled room, that have smooth sides and fully slatted floors. All pigs were fed their assigned diets in a daily amount of 3.2 times the estimated energy requirement for maintenance (i.e., 197 kcal metabolizable energy per $kg^{0.60}$; NRC, 2012). Two equal meals were provided every day at 0800 and 1700 h. Water was available at all times.

Pigs were fed experimental diets for 35 d. The initial 27 days were considered the adaptation period to the diets. On the last day of the adaptation period (i.e., on day 27), a blood sample was collected from the jugular vein of each pig via vena puncture. Blood was collected in ethylenediaminetetraacetic acid containing vacutainers. The collected samples were centrifuged at 1,500 × *g* at 4 ºC for 15 min, and plasma was collected and stored at -20 ºC until analyzed. Fecal samples were collected via anal stimulation during the following 4 days. Fecal samples were stored at -20 ºC until processed. Colon samples were collected from the colon cannulas for 9 h per day on days 32 and 33, and ileal digesta samples were collected from the ileal cannulas for 9 h per day on days 34 and 35. On days 32, 33, 34, and 35, the *ex-situ* pH was measured in

the first sample of colon or ileal digesta collected after 10 00 h using a pen pH electrode (Model ST20, Ohaus Corporation, Parsippany, NJ, USA).

Individual pig weights were recorded on day 1, on day 14, day 21, and at the conclusion of the experiment. At the conclusion of the experiment, colon and ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for chemical analysis.

Chemical Analyses

Fecal samples from each pig were thawed and dried in a forced-air drying oven (model Heratherm OMH750, Thermo Fisher Scientific Inc., Waltham, MA, USA) at 55 ˚C. Ileal digesta samples and colon digesta samples were lyophilized (model Gamma 1-16 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Diets, ingredients, dried ileal digesta, dried cecal digesta, and dried fecal samples were ground using a grain mill (500G Swing Type Grain Mill, RRH, Zhejiang, China) and mixed before analysis. Diets, ingredients, ileal digesta, cecal digesta, and fecal samples were analyzed for dry matter (method 930.15; AOAC Int., 2019), and for nitrogen using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as nitrogen × 6.25. Diets and ingredients were analyzed for ash (method 942.05; AOAC Int., 2019). Acid hydrolyzed ether extract was analyzed in diets and ingredients by acid hydrolysis using 3*N* HCl (AnkomHCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction [Method Am 5-04; AOCS, 2013] using petroleum ether (AnkomXT-15 Extractor, Ankom Technology, Macedon, NY). Diets and ingredients were analyzed for amino acids on a Hitachi Amino Acid Analyzer (Model L8800, Hitachi High Technologies America Inc., Pleasanton, CA, USA) using ninhydrin for post-column derivatization and nor-leucine as the internal standard. Samples were hydrolyzed with 6*N* HCl

for 24 h at 110 °C prior to analysis, but methionine and cysteine were analyzed as methionine sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis and tryptophan were determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E (a, b, c); AOAC Int., 2019]. Diets, ingredients, ileal digesta, cecal digesta, and fecal samples were analyzed for gross energy on a bomb calorimeter (Model 6400; Parr Instruments, Moline, IL, USA) using benzoic acid as the standard for calibration. Starch was analyzed in ingredient samples by the glucoamylase procedure (method 979.10; AOAC Int., 2019). Diets, ingredients, ileal digesta, cecal digesta, and fecal samples were also analyzed for IDF and SDF according to method 991.43 (AOAC Int., 2019) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of IDF and SDF. Diets, ileal digesta samples, colon digesta samples, and fecal samples were analyzed for titanium as well using inductively coupled plasma-optimal emission spectrometry (ICP-OES, Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600ºC for 4 hours (method 942.05, AOAC Int., 2019) and wet digestion with sulfuric acid (Myers, et al., 2004). The particle size of diets was determined using 100 g of the diet sample placed on top of test sieves that were placed in a vibratory sieve shaker for 15 min. The weight of the material in each of the test sieves was recorded for the calculation of mean particle size (ANSI/ASAE, 2008). Diets were also analyzed for bulk density as described by Navarro et al. (2018a), and diets and ileal digesta samples were analyzed for water binding capacity as described by Navarro et al. (2018a). Plasma samples were analyzed for peptide YY using an enzyme-linked immunosorbent assay kit according to recommendations from the manufacturer (MyBioSource Inc., San Diego, CA, USA). Diets were analyzed for xylanase and phytase activity using the QuantiPlate ELISA

kit specific for Econase XT and for Quantum Blue, respectively (ESC Standard Analytical Method SAM099 and SAM115; AB Vista, Plantation, FL, USA).

Calculations and statistical analyses

Values for AID, ACD, and ATTD of dry matter, crude protein, gross energy, IDF, SDF, and TDF were calculated as previously explained (Jaworski and Stein, 2017). Normality of data were verified and outliers were identified using the INFLUENCE statement, and the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified as values with internally studentized residuals greater than 3 or less than -3 (Tukey, 1977). A pig was excluded from statistical analysis if 3 or more response variables were identified as outliers. One pig fed the diet with xylanase was identified as an outlier and removed; however, two pigs fed the control diet, two pigs fed the diet with xylanase, and one pig fed the diet with xylanase and XOS lost a cannula or got sick during the experiment and were removed from the analysis. All other pigs were included in the final analysis. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included diet as main effect and pig blocked by body weight as random effect. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

There were no differences in the body weight of pigs during the experiment (Table 7.4). Concentrations of peptide YY in the plasma, pH in colon and ileal digesta, and the water binding capacity of ileal digesta of pigs were also not different among treatments. However, the AID of dry matter, gross energy, IDF, and TDF were greater ($P < 0.05$) in the diet containing xylanase or in the diet containing xylanase and XOS compared with the control diet (Table 7.5). The AID

of SDF tended to be greater ($P < 0.10$) in the diet containing xylanase than in the control diet. The diet containing xylanase had greater ($P < 0.05$) ACD of dry matter, gross energy, IDF, and TDF compared with the control diet, and the ACD of crude protein and SDF tended to be greater ($P < 0.10$) in the diet containing xylanase than in the control diet. The ATTD of dry matter, gross energy, and IDF was greater ($P < 0.05$) in the diet containing xylanase than in the control diet, and the diet containing xylanase had greater ($P < 0.05$) ATTD of TDF compared with the control diet and the diet containing xylanase and XOS.

DISCUSSION

Concentrations of dry matter, crude protein, amino acids, gross, starch, IDF, SDF, TDF, ash, and acid-hydrolyzed ether extract of ingredients were in agreement with reported values (NRC, 2012). Corn contains close to 5% arabinoxylans (dry matter-basis), and wheat middlings contain close to 22% arabinoxylans (dry matter-basis; Bach Knudsen, 2014; Jaworski et al., 2015). Therefore, with 44.80% corn in the diets, and 29% wheat middlings, the calculated concentration of arabinoxylans in the diets was around 9%, indicating that the diets used in this experiment provided the substrate for the xylanase. The analyzed nutrient composition of the diets used in the experiment were also in agreement with predicted values. The physical characteristics (i.e., particle size, bulk density, and water binding capacity) were not different among diets. The xylanase activity for the control diets did not exceed the detection limit (2,000 BXU/kg), and the analyzed xylanase activity for the diets with xylanase (26,100 BXU/kg) and xylanase and XOS (23,800 BXU/kg) was in agreement with the expected values.

Inclusion of wheat bran or wheat middlings in diets for pigs usually reduce AID and ATTD of nutrients and energy in diets for pigs due to the high fiber content (Huang et al., 2015;

Jaworski et al., 2016; Son et al., 2023). The improved AID of dry matter, gross energy, IDF, and TDF, in diets as xylanase or xylanase and XOS were included is in agreement with data indicating that addition of xylanase, or xylanase and XOS to diets for pigs increased digestibility of dry matter, energy, and fiber (Pedersen et al., 2015; Abelilla and Stein, 2019; Passos et al, 2015; Tiwari et al., 2018; Petry et al., 2019; 2021a). Xylanase hydrolyzes the β-(1–4) glycosidic bonds between 3 unsubstituted xyloses in the backbone of arabinoxylans, resulting in soluble small fractions of AXOS available to fermentation (Ravn et al., 2016; Tiwari et al., 2018). Xylo-oligosaccharides are fermentable sugar oligomers that have a stimbiotic effect on the gastrointestinal tract of pigs, by favoring growth of microbial populations that ferment fiber (Gonzalez-Ortiz et al., 2019; Cho et al., 2020). Results indicated that xylanase and the combination of xylanase and XOS can enhance energy and nutrient digestibility in the small intestine, which is in agreement with previous data (Petry et al., 2021a), which is likely a result of the ability of xylanase to release trapped nutrients in the fiber matrix by reducing the size of the fiber fractions and solubilize them, which then allow endogenous enzymes to access nutrients (Le et al., 2013). However, the lack of differences in water binding capacity of ileal digesta indicated that xylanase or xylanase and XOS do not impact physicochemical characteristics of digesta of pigs (Pettersson et al., 2013).

Although the *ex situ* pH of ileal digesta was not impacted by xylanase or xylanase and XOS, their effects in the small intestine may be related to fermentation and fast absorption of short-chain fatty acids as the digestibility of SDF tended to increase, which is in agreement with data reporting fermentation in the distal ileum of pigs (Petry et al., 2021a). Likewise, the pH of the digesta and cecal and colonic cells may be protected against pH changes due to increased concentration of short chain fatty acids through ion exchange mechanisms (Busche et al., 2002),

resulting in a constant pH regulation, which explains the lack of differences in the *ex situ* pH of colon digesta among treatments.

Because of the modes of action of xylanases and XOS, these additives are believed to increase digestibility of energy and nutrients. However, in this experiment, only xylanase increased hindgut digestibility. Increased ACD and ATTD of dry matter, gross energy, IDF, and TDF in diets with xylanase indicated that xylanase increases fermentation, which is in agreement with previous data (He et al., 2020; Petry et al., 2021; Galli et al., 2024; Acosta et al., 2025). The lack of differences in the ACD and ATTD of dry matter, gross energy, IDF, and TDF between the diet containing xylanase and XOS and the control is likely because the XOS added to the diet may have been fermented in the small intestine. The added XOS in diets may increase the abundance of *Lactobacillus* and *Bifidobacterium* in the ileal digesta, whereas AXOS may increase the abundance of *Lactobacillus, Bifidobacterium, and Faecalibacterium* in the colonic digesta (Petry et al., 2021b; Sun et al., 2023. Results also align with data indicating that observed that the effect of xylanase or XOS alone may change over time (Petry et al., 2024). Xylanase alone required between 14 and 27 days of adaptation to affect energy digestibility, but its impact became greater over time, whereas XOS alone showed results initially, but the benefit on energy produced by fermentation decreased after 28 days (Petry et al., 2020; 2024). Structural properties that differentiate AXOS produced by xylanase from XOS, such as the arabinose/xylose ratio, the degree of substitution with ferulic acid or monosaccharides, the presence of one or more arabinose side chains, and the degree of polymerization of the xylose backbone may impact the fermentability of AXOS and XOS in the hindgut of pigs (Dotsenko et al., 2018; Chen et al., 2024; Kulathunga and Islam, 2025). Therefore, the hypothesis that xylanase improve energy and fiber digestibility when included in diets containing wheat middlings was accepted. However, the

hypothesis that xylanase and XOS also increase digestibility was only partially accepted because only AID and not ACD and ATTD increased when xylanase and XOS were used. More research is warranted to describe the structure of AXOS produced by xylanase and to understand the differences in the mechanisms of action of AXOS produced by xylanase and XOS and their interaction with the microbiome in the gastrointestinal tract of pigs.

The lack of effects of xylanase or xylanase and XOS on concentrations of peptide YY in plasma is in agreement with data indicating no effects of xylanase and XOS on plasma peptide YY (Taylor et al., 2018), but in contrast to results of experiments where xylanase decreased or increased the concentration of peptide YY in plasma (Singh et al. 2012; May et al., 2015; Chen et al., 2020). This discrepancy may be due to differences in feed intake of pigs among experiments, or the composition of the diets. More research is needed to understand the impact of xylanase and XOS on hormonal regulation of feed intake in pigs.

CONCLUSIONS

Addition of xylanase increased the AID, ACD, and ATTD of dry matter, gross energy, IDF, and TDF in diets for pigs containing wheat middlings. These improvements are likely due to the enzymatic breakdown of arabinoxylans, which enhances nutrient accessibility by endogenous enzymes, and fiber fermentability. The combination of xylanase and XOS increased AID of dry matter, gross energy, IDF, and TDF in diets for pigs containing wheat middlings, but no ACD and ATTD of nutrients and energy, indicating that the combination of xylanase and XOS results in fermentation only in the distal ileum. Physicochemical properties of digesta, such as water binding capacity and pH were not influenced by xylanase or xylanase and XOS. Likewise, no differences in plasma peptide YY concentrations were observed by adding xylanase

or xylanase and XOS to the diets. Overall, xylanase alone was effective in enhancing nutrient and energy digestibility in diets for pigs containing wheat middlings.

TABLES

Table 7.1. Analyzed nutrient composition main of ingredients in diets (as-fed basis)

Item	Corn	Soybean meal	Wheat middlings
Gross energy, kcal/kg	3,866	4,153	4,020
Dry matter, %	85.58	88.23	89.66
Ash, %	1.26	5.87	5.95
Acid hydrolyzed ether extract, %	4.05	2.59	4.38
Crude protein, %	6.76	45.78	12.57
Starch, %	61.92	2.60	22.62
Insoluble dietary fiber, %	9.20	14.50	38.60
Soluble dietary fiber, %	0.70	3.70	2.60
Total dietary fiber, %	9.90	18.20	41.20
Indispensable amino acids, %			
Arg	0.34	3.29	1.07
His	0.21	1.22	0.44
Ile	0.25	2.21	0.47
Leu	0.84	3.61	0.92
Lys	0.23	2.95	0.67
Met	0.15	0.65	0.22
Phe	0.35	2.41	0.61
Thr	0.25	1.82	0.50
Trp	0.05	0.55	0.22

Table 7.1. (cont.)

Val	0.35	2.37	0.74
Dispensable amino acids, %			
Ala	0.53	2.03	0.73
Asp	0.50	5.21	1.07
Cys	0.16	0.66	0.36
Glu	1.35	8.57	2.70
Gly	0.28	1.95	0.85
Pro	0.62	2.28	0.86
Ser	0.32	2.04	0.59
Tyr	0.20	1.68	0.41

Table 7.2. Ingredient composition of experimental diets, as-fed basis

Ingredient, %	Control	Xylanase[1]	Xylanase and xylo-oligosaccharides[2]
Corn	44.80	44.80	44.80
Wheat middlings	29.00	29.00	29.00
Soybean meal	20.00	20.00	20.00
Choice white grease	2.70	2.70	2.70
Limestone	1.10	1.10	1.10
Enzyme premix[3]	1.00	1.00	1.00
Titanium dioxide	0.50	0.50	0.50
Salt	0.40	0.40	0.40
Vitamin-mineral premix[4]	0.50	0.50	0.50

[1]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK.

[2]Xylo-oligosaccharides = Short XOS 35A; AB Vista, Marlborough, UK.

[3]The premix for the control diet contained 50,000 phytase units (FTU)/kg of microbial phytase (Quantum-Blue 5G; AB Vista, Marlborough, UK; 0.1 kg phytase concentrate containing 5 million FTU/kg was mixed with 9.9 kg of ground corn). The premix for the xylanase diet contained 1.6 million BXU/kg of exogenous xylanase and 50,000 FTU/kg of exogenous phytase (0.1 kg xylanase concentrate containing 160 million BXU/kg and 0.1 kg phytase concentrate containing 5 million FTU/kg were mixed with 9.8 kg of ground corn). The premix for the xylanase diet and xylo-oligosaccharide contained 1.6 million BXU/kg of exogenous xylanase and 50,000 FTU/kg of exogenous phytase (0.1 kg xylanase concentrate containing 160 million BXU/kg, 0.1 kg phytase concentrate containing 5 million FTU/kg, and 0.05 kg xylo-oligosaccharides were mixed with 9.75 kg of ground corn).

Table 7.2. (cont.)

At 1% inclusion, the control diet was expected to contain 500 FTU/kg of phytase, and the diet with xylanase, and xylanase and xylo-oligosaccharides were expected to contain 500 FTU/kg of phytase and 16,000 BXU/kg of xylanase. BXU is the amount of enzyme that will release 0.06 µmoles of reducing sugars (xylose equivalents) from beechwood xylan per minute at pH 5.3 and 50 ºC. FTU is the amount of enzyme that will release 1 µmoles of inorganic phosphorus per minute at pH 5.5 from an excess of 15 M sodium phytate at 37°C. [4]The vitamin-micromineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

Table 7.3. Analyzed nutrient composition of experimental diets (as-fed basis)

Item	Control	Xylanase[1]	Xylanase and xylo-oligosaccharides[2]
Gross energy, kcal/kg	4,075	4,097	4,100
Dry matter, %	88.65	89.25	88.96
Ash, %	5.03	5.13	5.03
Acid hydrolyzed ether extract, %	7.62	7.81	7.62
Crude protein, %	17.36	17.42	17.42
Insoluble dietary fiber, %	17.20	18.50	20.00
Soluble dietary fiber, %	1.80	3.40	2.70
Total dietary fiber, %	19.00	21.90	22.70
Indispensable amino acids, %			
Arg	1.16	1.16	1.15
His	0.49	0.48	0.48
Ile	0.74	0.73	0.73
Leu	1.46	1.45	1.46
Lys	0.97	0.92	0.95
Met	0.28	0.27	0.27
Phe	0.88	0.87	0.88
Thr	0.67	0.67	0.66
Trp	0.17	0.19	0.19

Table 7.3. (cont.)

Val	0.91	0.89	0.89
Dispensable amino acids, %			
Ala	0.91	0.90	0.90
Asp	1.69	1.69	1.66
Cys	0.31	0.31	0.32
Glu	3.38	3.33	3.36
Gly	0.81	0.81	0.79
Pro	1.10	1.08	1.09
Ser	0.80	0.80	0.78
Tyr	0.58	0.57	0.57
Xylanase activity, BXU[3]/kg	< 2,000	26,100	23,800
Phytase activity, FTU[3]/kg	563	820	557
Bulk density, g/L	496.34	487.01	497.42
Water binding capacity, g/g	1.90	1.85	1.79
Particle size	446	478	442

[1]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK.

[2]Xylo-oligosaccharides = Short XOS 35A; AB Vista, Marlborough, UK.

[3]BXU is the amount of enzyme that will release 0.06 μmoles of reducing sugars (xylose equivalents) from beechwood xylan per minute at pH 5.3 and 50 ºC. The control diets did not exceeded the detection limit (2,000 BXU/kg). FTU is the amount of enzyme that will release 1 μmoles of inorganic phosphorus per minute at pH 5.5 from an excess of 15 M sodium phytate at 37°C.

Table 7.4. Body weight, digesta *ex situ* pH, water binding capacity of ileal digesta and concentrations of peptide YY in plasma of pigs fed experimental diets containing wheat middlings[1]

Item	Control	Xylanase[2]	Xylanase and xylo-oligosaccharides[2]	SEM	*P*-value
Body weight, kg					
D 1	46.13	52.84	48.77	2.95	0.328
D 14	55.00	63.66	57.66	4.12	0.371
D 21	62.03	73.12	64.83	4.73	0.288
D 35	71.63	84.88	75.43	5.42	0.267
Ex situ pH					
Ileal digesta	6.43	6.15	6.27	0.20	0.507
Colon digesta	5.71	5.91	5.82	0.17	0.617
Ileal digesta					
Water binding capacity, g/g	3.03	2.84	2.92	0.15	0.684
Peptide YY, pg/mL	97.59	43.16	50.01	21.58	0.187

[1]Data are least squares means of 6 observations for the control treatment, 5 observations for the xylanase treatment, and 7 observations for the xylanase and xylo-oligosaccharides treatment.

[2]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Xylo-oligosaccharides = Short XOS 35A; AB Vista, Marlborough, UK.

Table 7.5. Apparent ileal, cecal, and total tract digestibility of nutrients by pigs fed experimental diets containing wheat middlings[1]

Item	Control	Xylanase[2]	Xylanase and xylo-oligosaccharides[2]	SEM	*P*-value
Apparent ileal digestibility, %					
Dry matter	65.02^{b}	70.10^{a}	69.32^{a}	1.25	0.022
Gross energy	65.73^{b}	70.65^{a}	70.51^{a}	1.17	0.009
Crude protein	72.25	76.69	75.55	1.41	0.108
Insoluble dietary fiber	36.32^{b}	45.93^{a}	47.02^{a}	1.94	0.003
Soluble dietary fiber	0.02^{y}	27.82^{x}	21.82^{xy}	8.97	0.084
Total dietary fiber	31.82^{b}	43.68^{a}	43.80^{a}	2.30	0.002
Apparent cecal digestibility, %					
Dry matter	67.10^{b}	73.33^{a}	71.83^{ab}	1.51	0.029
Gross energy	65.52^{b}	72.62^{a}	71.08^{ab}	1.73	0.028
Crude protein	70.07^{y}	76.02^{x}	74.28^{xy}	1.63	0.062
Insoluble dietary fiber	38.14^{b}	47.08^{a}	43.35^{ab}	2.06	0.040
Soluble dietary fiber	46.91^{y}	66.39^{x}	62.96^{xy}	5.54	0.068
Total dietary fiber	39.10^{b}	49.48^{a}	45.90^{ab}	2.00	0.011
Apparent total tract digestibility, %					
Dry matter	78.41^{b}	80.52^{a}	79.45^{ab}	0.52	0.045
Gross energy	77.29^{b}	80.64^{a}	79.40^{ab}	0.77	0.028
Crude protein	82.55	84.33	82.91	0.94	0.424

Table 7.5. (cont.)

Insoluble dietary fiber	45.79[b]	51.08[a]	48.53[ab]	1.26	0.018
Soluble dietary fiber	74.19	80.40	71.88	3.82	0.242
Total dietary fiber	49.44[b]	54.64[a]	51.42[b]	1.20	0.006

[a-b]Values within a row lacking a common superscript letter differ ($P < 0.05$).

[x-y]Values within a row lacking a common superscript letter tend to differ ($P < 0.10$).

[1]Data are least squares means of 6 observations for the control treatment, 5 observations for the xylanase treatment, and 7 observations for the xylanase and xylo-oligosaccharides treatment.

[2]Xylanase = Econase XT 25 (160,000 BXU/g); AB Vista, Marlborough, UK; Xylo-oligosaccharides = Short XOS 35A; AB Vista, Marlborough, UK.

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CHAPTER 8: CONCLUSIONS

The overall objective of this research was to investigate the effects of exogenous enzymes and the combination of the enzyme xylanase with oligosaccharides (i.e., stimbiotic) on the utilization of dietary fiber, fermentation, digestibility of energy and nutrients, growth performance, and health of weanling and growing pigs. Because the use of fibrous ingredients is increasing in diets, and energy is the greatest and most expensive component of animal diets, additives such as enzymes and oligosaccharides are a strategy to increase the ability of the pig to utilize the energy associated with dietary fiber when high fiber co-products are used in diets. However, the fundamental issue is our poor understanding about dietary fiber, which is diverse among ingredients, and how enzymes, alone or combine with oligosaccharides, increase fiber fermentation, how it contributes to the energy status of pigs, and what their impacts are on the health of pigs.

It was essential to characterize the feed ingredients by using the enzymatic-gravimetric method to obtain total dietary fiber (**TDF**) as insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) fractions. The analysis of TDF is a robust and reproducible approach that provides valuable information to characterize fiber in most feed ingredients, except for oilseeds and oilseed coproducts due to oligosaccharides not accounted in the analysis. However, the information of the IDF and SDF in ingredients can be used to predict their energy value, as SDF is much more fermentable than IDF. Likewise, it can be used as a source of information about the IDF that can be utilized by enzymes as substrate.

Supplementation with a novel endo-β-mannanase was demonstrated to be safe, without compromising the health or growth of weanling pigs, even if an overdose was added to the diets. The supplementation of xylanase and β-glucanase combined in diets results in more

metabolizable energy by growing pigs, resulting in increased fiber fermentation by the microbiome in the intestinal tract of pigs. However, fiber fermentation may also result in microbial growth, and therefore, result in no impacts in the digestibility of fiber.

Feeding weanling pigs with diets containing xylanase or a stimbiotic improved energy digestibility during late nursery period, resulting in greater growth performance of pigs 42 days post-weaning. However, supplementing sows with xylanase during lactation did not affect the growth of the pigs after weaning as hypothesized. While stimbiotic supplementation increased the presence of nutrient transport proteins in the blood and xylanase reduced inflammatory cytokines, xylanase and stimbiotic did not influence intestinal morphology or the expression of tight junction proteins. This indicates that the improvements in digestibility and growth by xylanase and stimbiotics may be due to interactions among diet, the host, gut microbiota, physiological processes, and immune responses. However, xylanase or a stimbiotic can improve energy utilization, gut health, and overall performance in weanling pigs fed high fiber diets.

Addition of xylanase in high fiber diets for growing pigs improved the apparent ileal digestibility (**AID**), apparent colonic digestibility (**ACD**), and apparent total tract digestibility (**ATTD**) of gross energy, IDF, and TDF, likely due to the ability of xylanase to hydrolyze arabinoxylans, thereby increasing nutrient availability and promoting fiber fermentation. When a stimbiotic was used, improvements were observed only in the AID of gross energy, IDF, and TDF, but not in ACD or ATTD, suggesting that fermentation occurred in the small intestine. The physical characteristics of digesta, including water-binding capacity and pH, are not impacted by either xylanase alone or stimbiotic, and concentrations of peptide YY in plasma were not impacted either with the addition of xylanase or stimbiotic. Overall, xylanase increased fiber

fermentation when used in diets for growing pigs, whereas more research is needed to better understand the interaction of stimbiotics with the host and the intestinal microbiota.

Overall, results presented in this dissertation demonstrated that exogenous enzymes have positive effects on digestibility and fermentability across the gastrointestinal tract, as well as increase utilization of energy, growth performance and health of pigs fed high fiber diets. Future research should explore the interactions between other combinations of enzymes and oligosaccharides and further investigate microbiome-mediated mechanisms of enzymes and oligosaccharides across different fiber types used in diets for pigs.